PRMT BIOLOGY DURING ACUTE EXERCISE

EXERCISE-INDUCED PROTEIN ARGININE METHYLTRANSFERASE EXPRESSION, AND FUNCTION IN SKELETAL MUSCLE

By TIFFANY L. VANLIESHOUT, B.Sc. Kin Honours

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TITLE: Exercise-induced Protein Arginine Methyltransferase Expression and Function in Skeletal Muscle

AUTHOR: Tiffany L. vanLieshout, B.Sc. Kin Honours (McMaster University)

SUPERVISOR: Dr. Vladimir Ljubicic

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Lay Abstract

Skeletal muscle is a plastic tissue that can adapt to various physiological demands. Previous work suggests that protein arginine methyltransferases (PRMTs) are important in the regulation of skeletal muscle remodeling. However, their role in exercise-induced skeletal muscle plasticity is unknown. Therefore, the purpose of this study was to investigate the association between the intracellular signals required for muscle adaption and various metrics of PRMT biology. Our data demonstrate that PRMTs exhibit muscle-specific expression and function in mice. The movement of PRMT1 into myonuclei increased following exercise, while the specific methylation status of PRMT targets were also elevated. Overall, our data suggests that muscle-specific PRMT expression may be important for the determination and/or maintenance of different fiber type characteristics. Moreover, distinct PRMT cellular localization and methyltransferase activity may be key signals that contribute to skeletal muscle phenotypic plasticity.

Abstract

Protein arginine methyltransferase 1 (PRMT1), -4 (also known as coactivator-associated arginine methyltransferase 1; CARM1), and -5 catalyze the methylation of arginine residues on target proteins. In turn, these marked proteins mediate a variety of biological functions. By regulating molecules that are critical to the remodelling of skeletal muscle phenotype, PRMTs may influence skeletal muscle plasticity. Our study tests the hypothesis that the intracellular signals required for muscle adaptation to exercise will be associated with the induction of PRMT expression and activity. C57BL/6 mice were assigned to one of three experimental groups: sedentary (SED), acute bout of exercise (0PE), or acute exercise followed by 3 hours of recovery (3PE). The mice in the exercise groups performed a single bout of treadmill running at 15 m/min for 90 minutes. We observed that PRMT gene expression and global enzyme activity are muscle-specific, generally being higher in slow, oxidative muscle, as compared to faster, more glycolytic tissue. Despite the activation of canonical exercise-induced signalling involving AMPK and PGC-1α, PRMT expression and activity at the whole muscle level were unchanged. However, subcellular analysis revealed the exercise-evoked myonuclear translocation of PRMT1 prior to the nuclear translocation of PGC-1α, which colocalizes the proteins within the organelle after exercise. Acute physical activity also augmented the targeted methyltransferase activities of CARM1, PRMT1, and -5 in the myonuclear compartment, suggesting

that PRMT-mediated histone arginine methylation is an integral part of the early signals that drive skeletal muscle plasticity. In summary, our data supports the emergence of PRMTs as important players in the regulation of skeletal muscle plasticity.

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Be kind,

Try your hardest,

And have fun

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List of Abbreviations

0PE	0 hours post exercise
3PE	3 hours post exercise
ADMA	asymmetric dimethylarginine
ADP	adenosine diphosphate
AMP	adenosine monophosphate
AMPK	AMP-activated protein kinase
ATP	adenosine triphosphate
CaMKII	calmodulin-dependent protein kinase II
CARM1	co-activator-associated arginine methyltransferase
E2F1	E2F transcription factor 1
GAPDH	glyceraldehyde 3-phosphate dehydrogenase
GAST	gastrocnemius
H2B	histone 2B
JMJD6	Jumonji domain-containing 6
MAPK	mitogen-activated protein kinase
MAT	methionine adeonsyltrasnferase
MEF	mouse embryonic fibroblast
MEF2	myocyte enhancer factor-2
MMA	monomethylarginine
NF-κB	nuclear factor-kappa B

NRF-1	muscle respiratory factor-1
p38	p38 mitogen-activated protein kinase
p53	tumor suppressor protein p53
PGC-1α	(PPARγ) coactivator-1α
PPAR	peroxisome proliferator-activated receptor-γ
PRMT	protein arginine methyltransferase
QUAD	quadriceps
RIP140	receptor interacting protein 140
RPS11	ribosomal protein s11
SAH	s-adenosylhomocysteine
SAM	s-adenosylmethionine
SC	satellite cell
SDMA	symmetric dimethylarginine
SED	sedentary
SIRT1	sirtuin 1
SOL	soleus
t-tubules	transverse tubules
Tfam	mitochondrial transcriptional factor A

Declaration of Academic Achievement

Tiffany L vanLieshout was the principal contributor. Tania Tajik assisted with Western blotting. Derek W Stouth assisted with conceiving and designing the study. Vladimir Ljubicic assisted with conceiving and designing the study, as well as with writing the manuscript.

Review of the Literature

1. Introduction to Skeletal Muscle

Skeletal muscle accounts for ~40% of total mammalian body mass and has a wide variety of functions, including postural control and movement ([1,2]). Additionally, skeletal muscle plays a central function in the control of whole-body metabolism with roles in glycemic control, metabolic homeostasis, and respiration ([1–3].). Skeletal muscle cells, also known as skeletal muscle fibers or myofibers, are typically long, cylindrical, and multinucleated. The outer membrane of skeletal muscle fibers is called the sarcolemma, which has a series of invaginations known as transverse tubules (t-tubules) at regular intervals. These t-tubules allow action potentials to penetrate the cell quickly to facilitate muscle contractile activity.

Muscle contractions begin with a neuronal stimulation that spreads along the t-tubules. The depolarization of the t-tubules causes gated calcium ion channels in the sarcoplasmic reticulum to open allowing calcium ions to diffuse into the sarcoplasm. The sarcoplasm, which is composed of numerous myofibrils, contains two primary types of filaments that together make a highly-ordered unit called the sarcomere. It is here on the actin myofilament that the calcium ions bind to troponin. This in turn causes tropomyosin to move and exposes active sites on the actin myofibril. Once the active sites are exposed, the heads of myosin myofilaments, which are the other primary myofilament, bind to active sites and form cross-bridges. Energy stored in the myosin head is then used to move them, causing the actin myofilament to slide past the myosin myofilament and releasing adenosine

diphosphate (ADP) molecules from the myosin head. Next, adenosine triphosphate (ATP) molecules bind to the myosin heads, detaching them from actin and releasing the cross bridge. ATP is then hydrolyzed and the heads of myosin return to their resting position. At this point, if calcium is still attached to troponin, cross-bridge formation and movement are repeated. Altogether this process is known as actin-myosin cross bridge cycling and as stated by the sliding filament theory, powers skeletal muscle movement ([2]).

Mammalian skeletal muscles are comprised of several fiber types and are traditionally classified by myosin heavy chain (MHC) isoform composition ([2,4]). Fiber types are defined as slow- (type I) or fast-twitch (type II) ([2]). Fast muscle fibers can be further subclassified as type IIa and IIx in humans, while type IIa, IIx, and IIb are found in rodents ([2,5,6]). It is important to note that the structural and functional characteristics of skeletal muscle fibers exist along a spectrum, with type I and type IIb myofibers occupying the poles, while the remaining fiber types are situated in between. For example, type I fibers have a small cross-sectional area (CSA), high mitochondrial density, large satellite cell count, large neuromuscular junction size, and greatest number of capillaries per fiber, while type IIb fibers generally demonstrate the opposite characteristics ([2,5]). Furthermore, type I muscles are classically red in appearance while IIb are white, which reflects the relative abundance of capillaries, as well as the oxygen transport protein myoglobin, and mitochondrial content ([2]). Altogether, type I fibers are highly oxidative in nature,

and therefore possess the greatest endurance capacity, whereas in contrast type IIb fibers produce higher forces and contraction/relaxation velocities, and rely more heavily on glycolytic metabolism, thus fatiguing relatively quickly. Type IIa fibers, along with other hybrid myofibers, fall in-between these limits with respect to their molecular, biochemical, morphological, and functional characteristics.

While skeletal muscle can be generally be classified as either fast, glycolytic or slower, and more oxidative, the specific distribution of fiber types differs across various muscles. For example, in mice the extensor digitorum longus (EDL), soleus (SOL), and gastrocnemius (GAST) muscles have the following distributions of type I, IIa, IIx, and IIb myofibers: EDL = ~0%, ~19%, ~22%, ~47%; SOL = ~31%, ~50%, ~12%, ~3%; GAST = ~0%, ~21%, ~15%, ~56% ([6]). While these serve as a general range of muscle fiber composition, the relative proportions are plastic as muscle fibers can remodel their phenotype in response to various stimuli, such as disease, disuse, and exercise ([2]).

2. Skeletal Muscle Plasticity

Skeletal muscle is extremely malleable and can adapt to a multitude of intrinsic or extrinsic stimuli ([7–9]). For example, chronic endurance exercise training can induce a shift toward a slower, more oxidative phenotype that exhibits enhanced fatigue resistance ([10,11]). In contrast, prolonged muscle disuse causes atrophy and a shift in phenotype towards fast, glycolytic characteristics ([11,12]). The remodeling of skeletal muscle depends, in part, on the activation or inhibition of

various intracellular signaling pathways, which in turn alter the expression of genes that influence muscle structure and function ([2]). The increased expression and/or activity of molecules such as peroxisome proliferator-activated receptor (PPAR)-γ coactivator-1α (PGC-1α), PPARδ, tumor suppressor protein p53 (p53), p38 mitogen-activated protein kinase (p38), and AMP-activated protein kinase (AMPK) all play important roles in remodeling skeletal muscle toward a slower, more oxidative phenotype ([10,13–17]). On the other hand, receptor interacting protein 140 (RIP140), and E2F transcription factor 1 (E2F1) promote fast, glycolytic characteristics ([18–20]). It is the balance of the actions of these phenotype-bending proteins, and many others ([2,21]), that maintain and remodel skeletal muscle phenotype.

3. Bout of Acute Exercise

Skeletal muscle phenotype is incredibly plastic and can remodel in response to various stressors. It is well established that chronic muscle use evokes a phenotype shift in skeletal muscle. For example, endurance exercise training elicits slower, more oxidative characteristics in the active muscles ([21]). These adaptations improve the mechanical and metabolic efficiency of skeletal muscle, which in turn enhance fatigue resistance ([22]). Training-induced skeletal muscle plasticity begins with the discrete response to each exercise session. Although it alone is insufficient to elicit adaptations in muscle phenotype, the molecular response to an acute bout of physical activity is required for the stimulation of a muscle-remodelling program

([2,23]).

During contractile activity, a variety of intracellular signals are initiated including cyclical fluctuations in cytosolic Ca^{+2} concentration, alterations in the adenosine monophosphate (AMP):ADP/ATP ratio, as well as increased mitochondrial oxygen consumption and oxidant production ([2,22]). These alterations are sensed by signaling molecules such as calmodulin-dependent protein kinase II (CaMKII), AMPK, as well as the mitogen-activated protein kinase (MAPK) family ([2,21]). The activation of these molecules often stimulates acute alterations in muscle metabolism and/or function, as well as downstream gene expression programs that are crucial for adaptations to exercise. For example, during exercise a calcium-mediated increase in CaMKII phosphorylation is observed in an intensity-dependent manner ([24,25]), acutely influencing glucose transport ([26]), lipid uptake and oxidation ([27]), while chronically affecting skeletal muscle plasticity via the activation of downstream gene expression pathways mediated by CREB, myocyte enhancer factor-2 (MEF2), and HDACs ([2,28,29]).

Stimulation of downstream transcription factors and coregulators including PPARδ, nuclear respiratory factor-1 (NRF-1), MEF2, and PGC-1α ([2,10,30]) plays an important role in exercise-induced muscle remodelling. Regulation of these factors occurs via changes in protein content, subcellular localization, or activity through posttranslational modifications ([2]). For example, the transcriptional coactivator PGC-1α recruits and coregulates multiple transcription factors that regulate skeletal

muscle gene expression like MEF2, NRF-1, NRF-2 (also known as GA-binding protein), and the mitochondrial transcriptional factor A (Tfam) ([31]). A single bout of exercise increases PGC-1α gene expression and protein level ([32–34]), as well as induces its nuclear localization ([35,36]) in rodents and humans. PGC-1α activity is also tightly regulated by posttranslational modifications including phosphorylation by AMPK and p38, and deacetylation by SIRT1 ([37–39]). Wright and colleagues (Wright J Biol Chem 2007) demonstrated that exercise-induced mitochondrial biogenesis begins before the increase in PGC-1α protien levels ([34]), pointing to the importance of changes in PGC-1α activity and/or localization likely mediated by posttranslational modifications. Furthermore, numerous genetic and pharmacological studies have clearly demonstrated the importance of these transcription factors and coregulators to skeletal muscle remodeling ([2,21]). Sustained activation of a single enzyme, AMPK for example ([13,40]), evokes a robust shift in phenotype towards characteristics indicative of the slow, oxidative myogenic program, recapitulating certain adaptations elicited by endurance-type exercise training ([41]).

The period following exercise is characterized by two major phases: 1) the recovery of myocellular energy and ion homeostasis, and 2) induction of the adaptive cellular response to exercise ([2]). The various contraction-sensitive molecules discussed above display some shared, and some unique, expression and activity profiles during the recovery period. For instance, AMPK activation, as

indicated by phosphorylation of the alpha subunit at Thr172, increases immediately following physical activity and then decreases three hours post exercise in mouse skeletal muscle ([42–44]). On the other hand, PGC-1α mRNA levels have been shown to significantly increase immediately following exercise, as well as after 180 minutes of recovery ([36,45]). It is clear that a molecular response within the active muscles is observed immediately and shortly after an acute training stimulus. Thus, a comprehensive investigation of the mechanisms that mediate muscle plasticity in response to exercise should examine intracellular signaling and gene expression throughout the recovery period.

4. Introduction to PRMTs

4.1 General properties of PRMTs

The methylation of arginine residues on target proteins is known as arginine methylation. In mammals, this process occurs to the same extent as phosphorylation and ubiquitylation ([46,47]) and is carried out by the nine members of the protein arginine methyltransferase (PRMT) family ([48]). Through this process, PRMTs alter the stability, localization, and activity of their substrates ([47,49,50]), and thus these enzymes have emerged as critical regulators of a variety of biological functions including signal transduction, transcriptional activation and repression ([47,51–53])

PRMTs catalyze the transfer of a methyl group from S-adenosylmethionine (SAM) to the guanidine nitrogen atoms of arginine resulting in the formation of a methylarginine and side product of S-adenosylhomocysteine (SAH) ([54]). SAM is

generated by the enzyme methionine adenosyltransferase (MAT) using methionine and ATP ([55]). Both glycine- and arginine-rich (GAR) motifs and/or proline-, glycine-, and methionine (PGM)-rich regions of proteins are major targets for arginine methylation ([56]).

In eukaryotes three main forms of methylarginines have been identified: monomethylarginine (MMA), asymmetric dimethylarginine (ADMA), and symmetric dimethylarginine (SDMA). The ratio of steady-state arginine levels in mouse embryonic fibroblast (MEF) cells are 1500:3:2:1 for Arginine:ADMA:MMA:SDMA ([57]). PRMTs are classified into three categories based on their catalytic activity: type I, II, and III. Type I PRMTs, including PRMT1, -2, -3, -4 (also known as co-activator-associated arginine methyltransferase or CARM1), -6, and -8, are responsible for MMA synthesis and can further convert arginine to ADMA. Type II PRMTs, which include PRMT5, and -9, produce both MMA and SDMA. Both ADMA and SDMA reactions require MMA and L-arginine to occur. PRMT7 is a type III PRMT that only generates MMA, and is the sole member of the PRMT family that falls into this category (Figure 1) ([58]).

The majority of PRMTs are found in a vast array of tissues including brain, lung, liver and gallbladder, pancreas, gastrointestinal tract, kidney and urinary bladder, reproductive organs, bone marrow and immune system, muscle, adipose, and skin ([56,59]). PRMT8 is the only enzyme without this ubiquitous expression

Figure 1

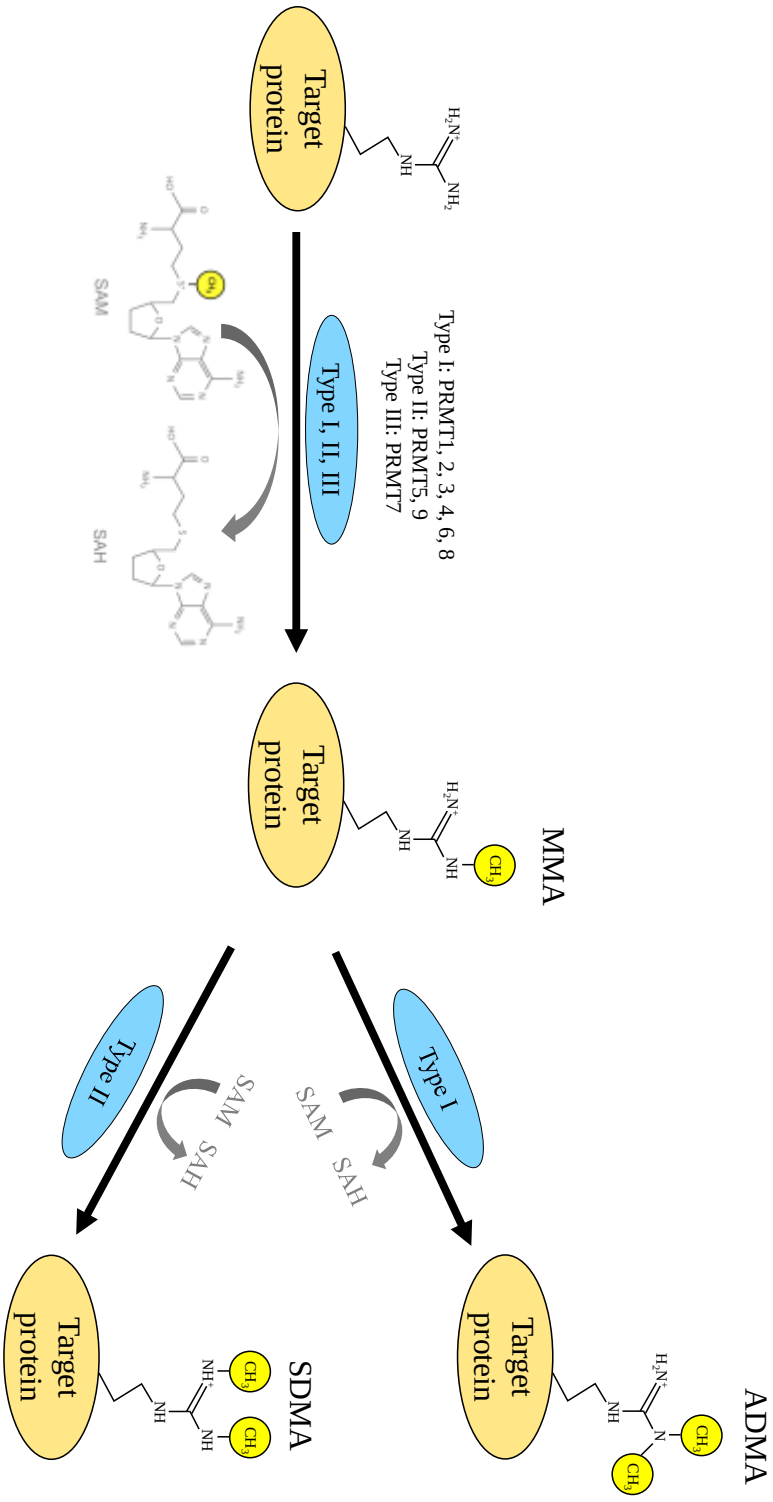
Target protein
Type I, II, III
Type I: PRMT1, 2, 3, 4, 6, 8
Type II: PRMT5, 9
Type III: PRMT7
SAM
SAH
MMA
Target protein
Type I
SAM SAH
Type II
SAM SAH
ADMA
Target protein
SDMA
Target protein

Figure 1: Protein arginine methyltransferase activity. Protein arginine methyltransferases (PRMTs) transfer a methyl group from S-adenosylmethionine (SAM) to the target protein and create the side product of S-adenosylhomocysteine (SAH). This enzyme family is classified into three categories based on their catalytic function: type I, II, and III. PRMTs in all three categories are responsible for the synthesis of the monomethylarginine (MMA) mark, with the type III enzyme PRMT7 having the sole responsibility of monomethylation. Both type I and II PRMTs monomethylate and dimethylate target proteins. Following monomethylation, type I PRMTs, which include PRMT1, -2, -3, -4, -6, and -8, deposit the asymmetric dimethylarginine (ADMA) mark, while type II PRMTs, including PRMT5 and -9, generate the symmetric dimethylarginine (SDMA) mark.

pattern as it is found primarily in the brain, with trace levels in muscle, the lungs, and reproductive organs ([54,59]).

It is important to note that the first known arginine demethylase, JMJD6 (or Jumonji-domain-containing 6), has been shown to demethylase both the symmetric and asymmetric forms of H4R3 ([60,61]). While further work is required to fully characterize JMJD6, this evidence points to the importance of methylation marks.

4.2 PRMT-specific expression and function

PRMT1

PRMT1 was the first mammalian protein arginine methyltransferase to be identified and is responsible for the majority (~85%) of total protein arginine methylation activity, as well as 50% of ADMA formation in MEFs ([47,62,63]). With its regulation by alternative splicing resulting in several isoforms, it has a wide specificity for substrates with a preference for residues that are flanked by one or more glycine residues ([47,54]). Key functions of PRMT1 include transcriptional coactivation, signal transduction, RNA splicing, and DNA repair ([58]). PRMT1 is present in both the cytoplasm and nucleus with a greater affinity for the nucleus in the majority of tissues ([59,64]).

Abnormal levels of PRMT1 have been shown to play a role in cancer, cardiovascular, pulmonary, and renal diseases, as well as aging ([54]). Complete loss of PRMT1 in mice results in an embryonic lethal phenotype ([49]). Interestingly, knockout or knockdown of PRMT1 in cell culture models leads to reduced ADMA

levels, which is accompanied by the compensatory upregulation of MMA and SDMA marks ([62]).

PRMT2

Only recently recognized as a Type I PRMT ([65]), PRMT2 functions as a transcriptional coactivator of both the androgen receptor and the estrogen receptor α ([66]). PRMT2 also promotes apoptosis and inhibits nuclear factor-kappa B (NF-κB) transcription though the blockage of IκB-α (nuclear factor of kappa light polypeptide gene enhancer in B-calls inhibitor, alpha) nuclear export ([67]). While found in both the cytoplasm and nucleus, it is predominantly expressed in the nucleus ([64,66]). Its expression is altered in hypoxic conditions, breast cancer, pulmonary inflammation and metabolic diseases ([47,54,58]). PRMT2 null mice are lean and resistant to dietary-induced obesity ([68]).

PRMT3

PRMT3 is predominantly located in the cytosol with trace levels present in the nucleus ([64]) and is unique for its zinc-finger at its N-terminus ([69]). PRMT3 primarily methylates ribosomal proteins and therefore functions in translational regulation ([70]). It has been shown to play a role in certain cancers and metabolic diseases, as well as in neuronal lineage determination ([54]). PRMT3 null mice demonstrate a developmental delay resulting in smaller size after birth, but achieve normal size by adulthood ([70]).

PRMT4/CARM1

CARM1 is present in both the nucleus and cytoplasm, with predominantly cytoplasmic localization ([64]). Its main functions include transcriptional coactivation and RNA splicing, which in turn significantly impact cell proliferation and differentiation ([54,58]). CARM1 has been shown to interact with factors required for chromatin remodeling during gene expression ([54,71]). Abnormal levels of this PRMT have been implicated in various cancers, metabolic diseases, as well as aging. ([54]). CARM1 knockout mice are born smaller than their wild-type littermates and die perinatally ([72]).

PRMT5

PRMT5 is the major type II PRMT and plays a role in signal transduction, transcriptional repression, and RNA splicing ([58]). PRMT5 is critical for the maintenance of pluripotency in mouse embryonic stem cells as it represses key differentiation genes ([73]). Originally thought to only be present in the cytoplasm, PRMT5 has recently been shown to also localize in the nucleus ([74]). Irregular levels of PRMT5 have been implicated in neurodegenerative diseases, diabetes, aging, and cancer ([54]). Complete loss of PRMT5 is incompatible with life in mice ([65]).

PRMT6

As a nuclear enzyme, PRMT6 is also characterized by its specificity for distinct methy-accepting substrates and by its automethylation activity ([47,64]). Its major functions include transcriptional repression and activation ([47]). PRMT6 has been shown to play a role in early development, cell proliferation, bladder and lung

cancer, neurodegenerative diseases, and aging ([54,56]). PRMT6 null mice do not exhibit obvious macroscopic defects and are viable ([75]). Through the generation of a transgenic mouse model that ubiquitously expressed PRMT6 fused to the hormone-binding portion of the estrogen receptor (ER), it was shown that PRMT6 co-activates NF-*κ*B, pointing to a role for PRMT6 in the inflammatory response ([76]).

PRMT7

Initially characterized as a type II methyltransferase, PRMT7 has been recently identified as a type III PRMT. Located primarily in the cytoplasm, but also found in the nucleus, it plays a role in embryonic stem cell pluripotency, male germline gene imprinting, and other brain-specific functions ([54,58,64]). Three independent transgenic PRMT7 alleles exist, and the viability of the PRMT7 null mice depends on the allele and genetic background ([77–79]). PRMT7 dysfunction has been implicated in metabolic diseases and cancer ([54]).

PRMT8

While the amino acid sequence of PRMT8 is closely related to PRMT1, it has very narrow tissue distribution, being limited mainly to the brain ([47,64,80]). Due to this localization, PRMT8 has brain-specific functions, such as regulating neuronal development ([58]). Indeed, PRMT8 expression was recently revealed to be important for the differentiation of mouse embryonic stem cells into a neuronal lineage ([81]). PRMT8 is overexpressed in various cancers and has been revealed to

be the most mutated of PRMTs in cancer genomes ([56]). Recent work has shown that PRMT8 null mice are viable and that PRMT8 is expressed in pluripotent stem cells ([81]).

PRMT9

Relatively little is known about PRMT9 and its role(s) in vivo has yet to be determined ([54,80]). PRMT9 is found primarily in the cytoplasm, with some nuclear localization ([58,82]). PRMT9 contains two catalytic domains with conserved sequences in the double E-loop important for substrate specificity and activity ([82,83]). Mutations within a catalytic domain can switch PRMT9 activity from catalyzing the deposition of the SDMA mark to the MMA mark instead ([83]). It has recently been suggested that PRMT9 dysfunction plays a role in neurodegenerative diseases ([54]).

5. PRMT Targets that Regulate Skeletal Muscle Phenotype

PRMTs may play a role in regulating tissue plasticity, in part, by altering the activity of several transcription factors and transcriptional co-regulator targets. Various PRMT-interacting molecules, including PGC-1α, E2F1, RIP140, and p53 are powerful regulators of skeletal muscle plasticity ([22]). However, links between PRMTs and these phenotypic modifiers have not yet been explicitly made in skeletal muscle. The following section discusses these interactions in other cell types and provides a rationale for continuing their investigation in skeletal muscle.

5.1 PGC-1α

PGC-1α is a transcriptional coactivator that interacts with multiple transcription factors to stimulate phenotype determination and remodelling programs in numerous tissues ([10]). In skeletal muscle, PGC-1α serves as a key regulator of the slow, oxidative myogenic program ([10]). For example, transgenic overexpression of PGC-1α specifically within skeletal muscle results in mitochondrial biogenesis, a fast-to-slow myosin shift, structural and functional alterations in the neuromuscular junction, as well as improvements in VO_{2max} and improved endurance capacity ([30,38,84,85]). Exercise is a robust physiological stimulus for PGC-1α expression and activity in the skeletal muscle of rodents and humans ([86,87]).

Teyssier and colleagues first discovered a functional association between PRMT1 and PGC-1α when they demonstrated in CV-1 kidney cells that PRMT1 methylates the coactivator ([88]). PRMT1-mediated methylation of PGC-1α augmented PGC-1α transcriptional activity and mitochondrial biogenesis. Interestingly, the methylation of PGC-1α in CV-1 cells was PRMT1-specific, as the authors found that CARM1 did not enhance of the coactivator function of PGC-1α. This research linked together the most active PRMT with a master regulator of skeletal muscle phenotype. In doing so, it paved the way for further examination of the role of PRMTs in governing skeletal muscle plasticity.

5.2 p53

p53 plays a role in cell metabolism, growth and development ([89]). Endurance-type exercise localizes p53 to the mitochondria in skeletal muscle where it induces Tfam transcriptional activity and promotes organelle biogenesis ([90]). Work from the Roeder laboratory demonstrated the involvement of PRMT1 and CARM1 in p53 activation ([91]). Utilizing in vitro techniques with H1299 lung carcinoma cells and U2OS osteosarcoma cells, the authors very elegantly demonstrated the cooperative functions of p300, PRMT1, and CARM1 in transcriptional activation by p53. Furthermore, Jansson et al. demonstrated that PRMT5-mediated arginine methylation also regulates p53 function ([92]).

5.3 E2F1

E2F1 regulates the expression of genes involved in cell proliferation and participates in the control of cell cycle progression ([93]). Whole body E2F1 knockout animals demonstrate a highly oxidative phenotype, characterized by the increased expression of slower myosin isoforms, mitochondrial biogenesis, and enhanced fatigue resistance ([19]). In MEF cells, PRMT2 represses E2F1 transcriptional activity in a manner dependent on its interaction with the retinoblastoma gene product ([94]). In addition, Frietze and colleagues demonstrated that CARM1 is required for the estrogen-induced expression of E2F1 in a breast cancer cell line ([95]). These studies indicate that E2F1 activity is in part regulated by PRMTs, providing further linkage between PRMT-mediated arginine methylation and skeletal muscle plasticity.

5.4 RIP140

RIP140 is a transcriptional corepressor for many nuclear receptors and transcription factors ([96–99]) and plays an important role in the regulation of skeletal muscle phenotype and metabolism, in part by the suppression of phenotype modifying proteins such as PPARδ ([18]). RIP140 is expressed in a fiber type-specific manner, with low levels of the molecule associated with a greater abundance of oxidative myofibers. Utilizing a variety of complementary cell lines including COS-1, HEK293, 3T3-L1, and RIP140-null MEF, Wei's laboratory demonstrated that arginine methylation suppresses RIP140 activity largely via two mechanisms. First, PRMT1-mediated methylation of RIP140 attenuates its interactions with histones ([100]). Second, methylation of the corepressor by PRMT1 promotes its nuclear export ([100]). While PRMT2, -3, and -4 were all shown to interact with RIP140, PRMT2 and PRMT3 could also modulate the repressive activity of RIP140 while PRMT4 could not pointing towards individual PRMTs having different substrate preferences ([100]).

In summary, we have identified numerous regulators of skeletal muscle phenotype maintenance and remodelling, including PGC-1α, p53, E2F1, and RIP140, which are affected by PRMTs in other cell types. As the occurrence of protein arginine methylation is on par with that of phosphorylation or ubiquitylation ([46]), it is reasonable to assume that there are many additional targets of PRMTs that can mediate muscle plasticity. Thus, continued investigation of the general role(s) of PRMTs in muscle, and more specifically their protein targets, is warranted.

6. PRMT Research in Skeletal Muscle

6.1 Cell culture studies

Investigations of PRMT biology in skeletal muscle commenced shortly after the turn of the 21st century. The first work to examine PRMTs in skeletal muscle came from George Muscat's laboratory in 2002. Their research employing the C_2C_{12} model of skeletal muscle myogenesis demonstrated that CARM1 potentiates skeletal muscle differentiation, and potentiates myocyte maturation through its interaction with GRIP-1 to stimulate the activity of myocyte enhancer factor-2C (MEF2C) ([101]). Subsequently, Dacwag and colleagues demonstrated that PRMT5 facilitates myoblast proliferation, which is characteristic of the early stages of myogenesis ([102]). PRMT5 facilitates the myogenic program, in part, via a mechanism involving Brg-1-dependent chromatin remodeling and gene activation ([102]). About the same time as this discovery, Iwasaki and Yada demonstrated that PRMT1 mediates insulin-stimulated glucose uptake in L6 myocytes. The authors found that siRNA-mediated knockdown of PRMT1 expression resulted in attenuated insulin-stimulated IR/IRS-1/P13-K signaling and glucose disposal ([103]). Following up on their earlier work, Dacwag et al revealed that while both CARM1 and PRMT5 promote myogenesis, each enzyme displays a unique temporal influence on this process. CARM1 is required for late- but not for early-gene expression, while PRMT5 is required for early-gene induction but dispensable for late-gene expression ([104]). These data are in line with the findings of Chen and

colleagues (2002) with respect to the role of CARM1 in the later stages of skeletal muscle differentiation as well as its interaction with MEF2. Finally, Kim et al demonstrated that cellular ADMA levels, an indication of global type I PRMT activity, were altered during the process of myoblast fusion ([105]). PRMT1-specific catalytic activity, assessed by measuring the methylation of hnRNPA1, was highest during and after fusion. In contrast, the pattern of SDMA-marked proteins remained consistent throughout the transition from myoblasts to myotubes, while PRMT5-specific activity, evidenced by MBP methylation, peaked after myoblast fusion. The researchers also found that PRMT-mediated symmetric and asymmetric dimethylation of the myonuclear protein lamin A/C is important for muscle development and maintenance ([105]). Thus, these early, in vitro studies employing muscle cell cultures collectively demonstrated a clear role for PRMTs in the remodelling of skeletal muscle during myogenesis.

6.2 In vivo studies

Ljubicic et al performed the initial investigation of PRMTs in mammalian skeletal muscle in vivo in 2012. The authors first demonstrated the presence of PRMT1 and CARM1 in adult muscle ([40]). They also observed that both the transcript and protein levels of these enzymes were differentially expressed, and that acute and chronic conditions of muscle remodelling, namely exercise and dystrophy, respectively, altered PRMT1 and CARM1 gene expression. This study was followed by more work from Muscat's laboratory, which examined the relative

transcript levels of PRMTs 1-6 in mouse skeletal muscle. Analysis of PRMT mRNA content in muscles of varying fiber type composition, including the quadriceps (QUAD), soleus (SOL), and gastrocnemius (GAST) muscles, revealed fiber type-specific expression patterns of PRMT mRNAs. In the QUAD and SOL muscles, CARM1 was the most abundant transcript, followed by PRMT5 and PRMT1 ([106]). In contrast, CARM1 and PRMT5 were expressed at similar levels in the GAST, followed by PRMT1 ([106]). Cumulatively, these early in vivo studies demonstrated the presence of PRMTs in adult skeletal muscle and provided the first examples of PRMT gene expression during conditions of skeletal muscle plasticity.

A series of papers followed that examined the roles of PRMT1, CARM1, PRMT5, and PRMT7 in regulating skeletal muscle regeneration and repair in response to cytotoxic injury ([107–110]). Recent, excellent surveys by Blanc and Richard summarize the contributions of these PRMTs to the in vivo myogenesis process ([48,54]). In short, Kawabe et al demonstrated that arginine methylation of Pax7 by CARM1 functions as a molecular switch controlling the induction of Myf5 during satellite cell asymmetric division and entry into the myogenic program ([107]). Work investigating the role of PRMT5 in muscle stem cells found that PRMT5 generates a ready state that keeps muscle satellite cells in standby, allowing rapid amplification when needed ([108]). Furthermore, muscle stem cell fate is regulated, in part, through PRMT1-mediated arginine methylation within the Eya1/Six1/MyoD axis ([110]). Finally, PRMT7 has been shown to be a regulator of the DNMT3b/p21

axis which is required to maintain muscle stem cell regenerative capacity (Figure 2) ([109]).

In addition to mediating muscle satellite cell biology, Jeong and colleagues (2016) recently demonstrated that PRMT7 is a key regulator of the slow, oxidative myogenic program. Muscles from whole body PRMT7 KO animals exhibit decreased oxidative metabolism concomitant with reduced expression of genes important for maintaining the slower, more oxidative phenotype, such as PGC-1α ([78]). These mice display an attenuated endurance exercise capacity compared to their WT littermates, as well as decreased energy expenditure. Through a series of elegant experiments, the authors demonstrated that the mechanism by which PRMT7 regulates the slow, oxidative phenotype involves its interactions with the p38/ATF2/PGC-1α pathway, resulting in enhanced PGC-1α expression and activity ([78]). Collectively, while in vivo research elucidating the expression and function of PRMTs in muscle is still limited, recent work has clearly demonstrated the emerging importance of this family of enzymes as regulators of skeletal muscle plasticity (Figure 3).

7. Study Objectives

Recent evidence strongly suggests that the PRMT family of enzymes are important players in the regulation of skeletal muscle plasticity. The most widely studied PRMTs are PRMT1, CARM1, and PRMT5 as together these molecules account for the majority of cellular arginine methylation reactions ([65]). PRMT1,

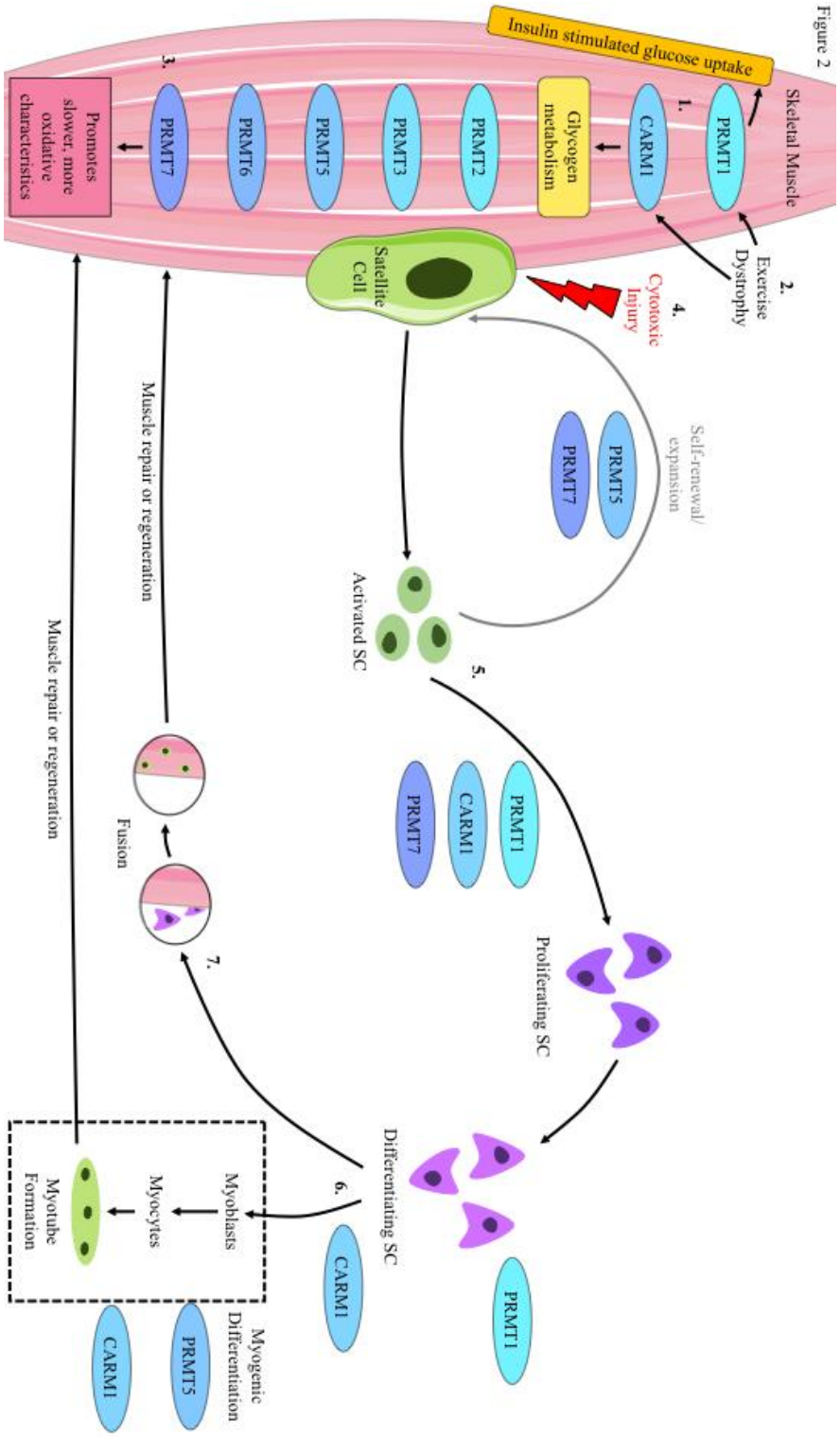
Figure 2
Skeletal Muscle
Insulin stimulated glucose uptake
PRMT1
CARM1
Glycogen metabolism
PRMT2
PRMT3
PRMT5
PRMT6
PRMT7
Promotes slower, more oxidative characteristics
1.
2.
Exercise
Dystrophy
3.
4.
Cytotoxic Injury
Satellite Cell
Self-renewal/ expansion
PRMT7
PRMT5
Activated SC
5.
PRMT1
CARM1
PRMT7
Proliferating SC
Differentiating SC
6.
CARM1
PRMT1
7.
Fusion
Muscle repair or regeneration
Muscle repair or regeneration
Myoblasts
Myocytes
Myotube Formation
Myogenic Differentiation
PRMT5
CARM1

Figure 2: PRMT expression and function in skeletal muscle. PRMT1-7 are present within skeletal muscle at varying amounts depending, in part, on fiber type composition. *1.* PRMT1 mediates insulin signalling and glucose disposal in skeletal muscle, while CARM1 is important for the regulation of glycogen metabolism. *2.* PRMT1 and CARM1 transcripts are induced in response to exercise and muscular dystrophy. The protein content of these enzymes is also augmented in the muscle of mdx mice, a pre-clinical model of Duchenne muscular dystrophy. *3.* PRMT7 regulates the slow, oxidative myogenic program, as its absence results in the expression of faster, more glycolytic characteristics. *4.* The majority of PRMT research in skeletal muscle has examined their expression and function within the context of muscle repair and regeneration in response to cytotoxic injury via cardiotoxin (CTX) administration. In response to CTX, satellite cells (SCs) exit quiescence to enter the proliferative state and undergo expansion. PRMT5 and -7 are important during this process as they promote SC symmetric division and renewal. *5.* CARM1, PRMT1, and -7 are critical for SC asymmetric division and further proliferation. PRMT1 is also important for terminal differentiation. *6.* CARM1 facilitates myogenic differentiation, while PRMT5 is important for early myogenesis and CARM1 plays a role in the formation of mature myotubes. These cells aid in muscle repair or regeneration. *7.* Alternatively, following differentiation SCs may fuse to existing myofibers to aid muscle repair or regeneration. Thus,

PRMTs reside in skeletal muscle, their expression levels are modifiable, and they have important roles to play in muscle and SC biology.

Figure 3 Influence: Positive → Negative ⊣

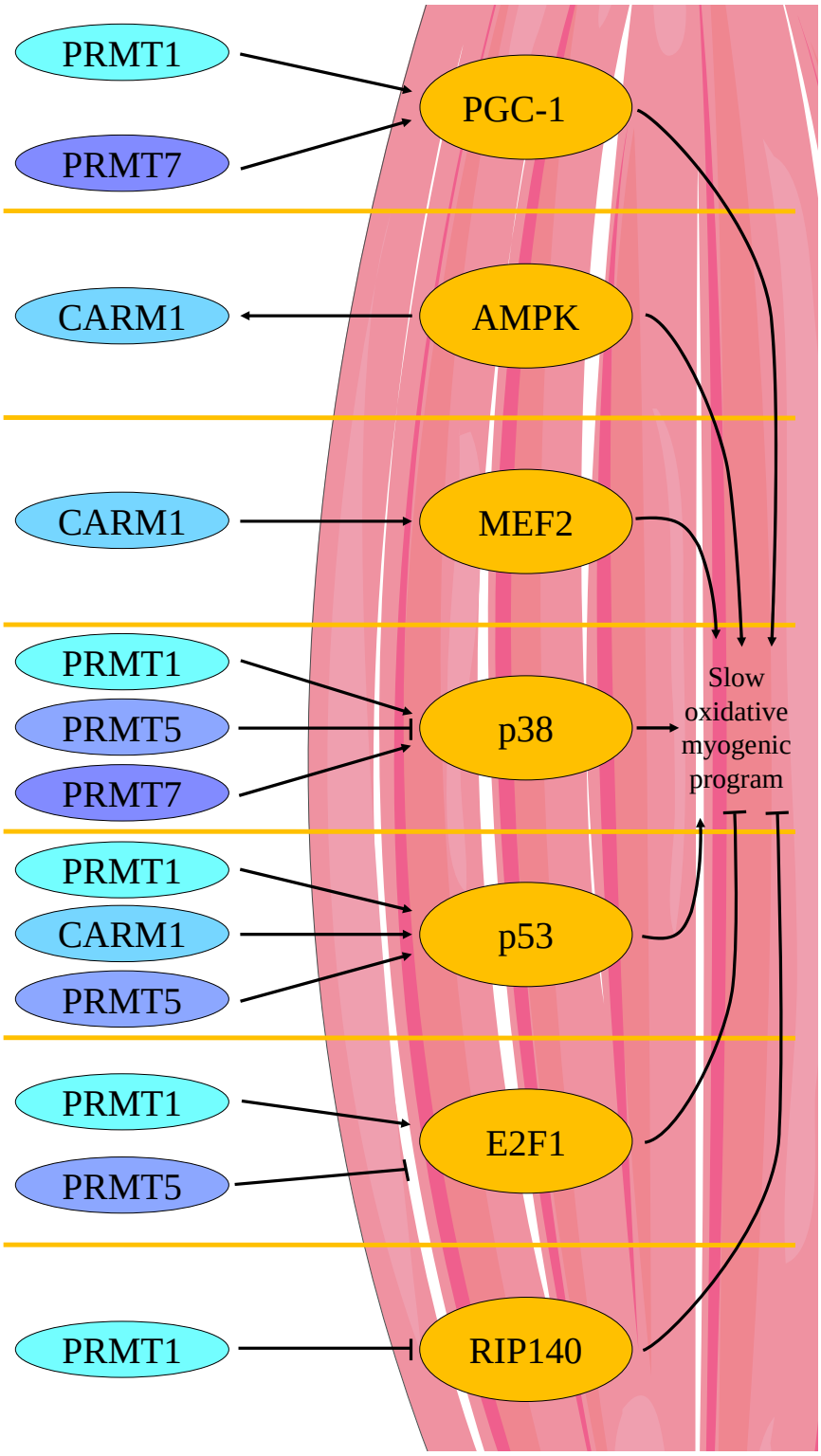

Figure 3: Proposed model for PRMT-mediated regulation of skeletal muscle plasticity. A limited number of in vitro and in vivo studies in skeletal muscle, as well as several other investigations in non-muscle tissues provide the rationale for this model of PRMT-mediated control of muscle remodelling. CARM1, PRMT1, -5, and -7 target proteins that govern the maintenance and plasticity of skeletal muscle phenotype. For example, PGC-1α activity is enhanced by PRMT1 and -7. CARM1 is in a signalling axis with AMPK, and CARM1 directly influences MEF2 in muscle. p38 is stimulated by PRMT1, and -7 while PRMT5 inhibits its activity . p53 is activated by CARM1, PRMT1, and -5. PRMT5 inhibits E2F1, while PRMT1 activates the molecule. RIP140 is inhibited by PRMT1. Chronic activation of PGC-1α, AMPK, MEF2, p38, and p53 cause a shift in skeletal muscle phenotype towards the slow, oxidative myogenic program, whereas in contrast E2F1 and RIP140 promote faster, more glycolytic characteristics.

CARM1, and PRMT5 play critical roles in the muscle regeneration process in response to cytotoxic injury ([48,54]). However, characteristics of PRMT biology during various other, physiological conditions of acute, adaptive skeletal muscle remodelling, such as exercise, are unknown. Addressing this knowledge gap will aid in furthering our understanding of the mechanisms that regulate phenotypic plasticity. Thus, the purpose of this Thesis was to characterize PRMT expression and function in skeletal muscle during acute muscle remodelling in vivo.

We hypothesized that induction of PRMT1, CARM1, and PRMT5 expression and activity would occur coincident with the intracellular signals associated with muscle adaptation to exercise.

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Exercise-induced Protein Arginine Methyltransferase Expression and Function in Skeletal Muscle

Tiffany L. vanLieshout, Tania Tajik, Derek W Stouth, and Vladimir Ljubicic

Department of Kinesiology, McMaster University, Hamilton, Ontario, Canada, L8S4L8

Abstract

Protein arginine methyltransferase 1 (PRMT1), -4 (also known as coactivator-associated arginine methyltransferase 1; CARM1), and -5 catalyze the methylation of arginine residues on target proteins. In turn, these marked proteins mediate a variety of biological functions. By regulating molecules that are critical to the remodelling of skeletal muscle phenotype, PRMTs may influence skeletal muscle plasticity. Our study tests the hypothesis that the intracellular signals required for muscle adaptation to exercise will be associated with the induction of PRMT expression and activity. C57BL/6 mice were assigned to one of three experimental groups: sedentary (SED), acute bout of exercise (0PE), or acute exercise followed by 3 hours of recovery (3PE). The mice in the exercise groups performed a single bout of treadmill running at 15 m/min for 90 minutes. We observed that PRMT gene expression and global enzyme activity are muscle-specific, generally being higher in slow, oxidative muscle, as compared to faster, more glycolytic tissue. Despite the activation of canonical exercise-induced signalling involving AMPK and PGC-1α, PRMT expression and activity at the whole muscle level were unchanged. However, subcellular analysis revealed the exercise-evoked myonuclear translocation of PRMT1 prior to the nuclear translocation of PGC-1α, which colocalizes the proteins within the organelle after exercise. Acute physical activity also augmented the targeted methyltransferase activities of CARM1, PRMT1, and -5 in the myonuclear compartment, suggesting

that PRMT-mediated histone arginine methylation is an integral part of the early signals that drive skeletal muscle plasticity. In summary, our data supports the emergence of PRMTs as important players in the regulation of skeletal muscle plasticity.

Introduction

Skeletal muscle phenotype is extraordinarily plastic and can remodel in response to various stressors. One important stimulus is chronic muscle use, which is well known to cause a phenotype shift in skeletal muscle. For example, endurance exercise training of appropriate intensity and duration elicits slower, more oxidative characteristics in the active muscles ([1]). These adaptations improve the mechanical and metabolic efficiency of skeletal muscle, which in turn enhance fatigue resistance ([2]). Training-induced skeletal muscle plasticity begins with the discrete response to each exercise session. The molecular response to an acute bout of physical activity is required for the stimulation of a muscle-remodelling program, however it alone is insufficient to elicit adaptations in muscle phenotype ([3,4]). A variety of intracellular signals are initiated during contractile activity, including cyclical fluctuations in cytosolic Ca^{2+} concentration, alterations in the AMP:ADP/ATP ratio, as well as increased mitochondrial oxygen consumption and oxidant production ([3,2]). These changes are sensed by signaling molecules such as Ca^{2+}/calmodulin-dependent protein kinase II (CMKII), AMP-activated protein kinase (AMPK), as well as the mitogen-activated protein kinase family ([3,5]). These

kinases stimulate the activity of downstream transcription factors and coregulators including peroxisome proliferator-activated receptor δ (PPARδ), nuclear respiratory factor-1 (NRF-1), myocyte enhancer factor 2 (MEF2), and PPARγ coactivator-1α (PGC-1α) ([3,6,7]). The importance of these molecules to skeletal muscle remodelling is established by genetic and pharmacological studies where chronic activation of a single enzyme, AMPK for example ([8,9]), evokes a robust shift in phenotype towards characteristics indicative of the slow, oxidative myogenic program, recapitulating certain adaptations elicited by exercise training ([10]). Despite this evidence however, a complete understanding of the molecules involved in regulating skeletal muscle plasticity has not yet been achieved.

Protein arginine methyltransferases (PRMTs) are a family of enzymes that catalyze the methylation of arginine residues on target proteins, thus altering the stability, localization and activity of their substrates ([11–13]). As the occurrence of arginine methylation is on par with that of phosphorylation and ubiquitylation ([14]), PRMTs have emerged as critical regulators of a variety of biological functions including signal transduction, transcriptional activation and repression ([6,11–13,15]). Type I, type II, and type III PRMTs are able to catalyze the addition of a single methyl group on the terminal nitrogen atom of a target protein to form a monomethylarginine (MMA) mark. PRMT7 is the sole member of the type III PRMT family and can only generate MMA. Type I PRMTs perform a second methylation step through the asymmetric dimethylation of an arginine residue,

which produces the asymmetric dimethylarginine (ADMA) mark and include PRMT1, -2, -3, -4 (also known as CARM1), -6, and -8. Type II PRMTs also perform a second methylation that is symmetric in nature and results in the formation of symmetric dimethylarginine (SDMA) and include PRMT5 and -9 ([15–17]). The most widely studied PRMTs are PRMT1, PRMT4, also known as co-activator-associated arginine methyltransferase 1 (CARM1), and PRMT5. Together, these molecules account for the majority of cellular arginine methylation reactions ([18]), with PRMT1 alone responsible for ~85% ([11,19,20]). Furthermore, whole body genetic deletion of PRMT1, CARM1, or PRMT5 leads to lethal phenotypes in mice ([19,21,22]). These data collectively demonstrate the vital role that these proteins play in protein regulation, cellular physiology, and survival.

PRMT1, CARM1, and PRMT5 regulate tissue plasticity, in part, by altering the activity of various transcription factor and transcriptional coregulator targets. For example, in CV-1 cells PRMT1 methylates PGC-1α, which augments PGC-1α activity and results in the induction of genes encoding mitochondrial proteins ([23]). PGC-1α, and other molecules targeted by PRMTs including receptor-interacting protein 140 (RIP140), p53, and E2F transcription factor 1 (E2F1), are powerful mediators of skeletal muscle plasticity ([24–26]). Thus, it is reasonable to postulate that PRMTs may mediate skeletal muscle phenotype maintenance and remodelling. Studies of PRMT expression and function in skeletal muscle, while limited, underscore the importance of these enzymes in regulating muscle biology. Initial in

vitro studies demonstrated critical roles for PRMT1, CARM1, and PRMT5 in numerous aspects of skeletal muscle morphology and function, including myogenesis, as well as insulin signalling and glucose metabolism ([27–30]). More recent, in vivo research has extended and expanded on these earlier studies. Ljubicic and colleagues were the first to demonstrate the presence of PRMT1 and CARM1 in adult, mammalian skeletal muscle ([31]). The authors observed that the transcript and protein levels of these enzymes were differentially expressed in muscle, and that acute and chronic conditions of muscle remodelling, namely exercise and dystrophy, respectively, altered PRMT1 and CARM1 gene expression. Subsequently, George Muscat's laboratory demonstrated that transcript levels of PRMTs 1-6 exhibit enzyme-specific expression patterns in skeletal muscles of varied fiber type composition ([32]). Furthermore, it was recently demonstrated that PRMT1, CARM1, PRMT5, and PRMT7 mediate muscle stem cell fate in vivo ([33–36]), while whole body ablation of PRMT7 resulted in a shift toward faster, more glycolytic characteristics in skeletal muscle ([37]). Collectively, the evidence suggests that PRMTs are novel regulators of skeletal muscle phenotype.

Although skeletal muscle PRMT biology has emerged as an area of intensified research focus, a more comprehensive examination of PRMT expression and activity during conditions of skeletal muscle remodelling would advance our understanding of the mechanisms that regulate phenotypic plasticity. Therefore, the purpose of this study was to characterize PRMT expression and function in skeletal

muscle during acute muscle remodelling in vivo. We employed a single bout of exercise and recovery in order to stimulate intracellular signalling associated with alterations in muscle phenotype. We hypothesized that induction of PRMT1, CARM1, and PRMT5 expression and activity would occur coincident with the intracellular signals associated with muscle adaptation to exercise.

Methods

Acute exercise protocol. All protocols were approved by the Animal Research Ethics Board at McMaster University and were carried out according to the regulations of the Canadian Council on Animal Care. Male C57BL/6 mice (n = 15-16; ~28.5 g body weight; 16-weeks-old) were purchased from Jackson Laboratory (Bar Harbor, ME). Mice were housed in an environmentally controlled room (23 °C, 12-hour light/12-hour dark cycle) and provided food and water ad libitum. Animals were randomly assigned to sedentary (SED), 0 hours post exercise (0PE), or 3 hours post exercise (3PE) groups. Animals in both the 0PE and 3PE groups were acclimatized to a rodent treadmill (i.e., 5 meters/minute for 5 minutes; Columbus Instruments, Columbus, OH) a day prior to the beginning of the experiment. The exercise protocol consisted of a single bout of treadmill running at 15 m/min for 90 min, similar to previous studies ([38,39]). The SED mice were killed by cervical dislocation while at rest, whereas the 0PE animals were killed immediately following exercise while the 3PE mice were returned to their cages and killed 180 min following the end of exercise. Extensor digitorum longus (EDL),

soleus (SOL), and gastrocnemius (GAST) muscles from one leg were rapidly excised, plunged into liquid nitrogen, and stored at -80 °C until subsequent processing. The EDL and SOL muscles from the opposite leg were embedded in optimal cutting temperature compound (OCT; Fisher Healthcare, Ottawa), frozen in liquid nitrogen-cooled isopentane, and stored at -80 °C.

RNA isolation and real-time polymerase chain reaction (RT-qPCR). Total RNA was isolated from frozen EDL and SOL muscles, as described previously ([9]). Briefly, muscles were homogenized in Trizol (Invitrogen, Carlsbad, CA) followed by purification and elution with the E.Z.N.A. RNA Isolation Kit (Omega, Mississauga, Canada). RNA concentration and integrity (i.e., A_{260}/A_{230}) were measured using a Nanodrop instrument (Thermo Scientific, Toronto) before being reverse transcribed into cDNA. Reverse transcription was performed as per the manufacturer's protocol provided with the cDNA Reverse Transcription Kit (Fisher Scientific, Toronto). RT-qPCR (Eppendorf, Hamburg, Germany) was performed with SYBR Green qPCR Master Mix (Qiagen, Hilden, Germany). qPCR data was transformed and analyzed using the delta delta CT method in order to quantify expression of various genes of interest relative to ribosomal protein S11 (RPS11) which served as the internal control gene (18). The primers used in this study were: PGC-1α forward (F) 5'- GGTGTAGCGACCAATCGGAA -3', reverse (R) 5'- TCACCAAACGCACGTCAGTA -3', PRMT1 F 5'- GGCTGAGGACCCTCTGGTAA -3', R 5'- GCTGGAGGCTCATCCCATTAG -

3’, CARM1 F 5’- CAACAGCGTCCTCATCCAGT -3’, R 5’- GTCCGCTCACTGAACACAGA -3’, PRMT5 F 5’- TCTCCCCACCAGCATTTTCC -3’, R 5’- TGGAGGGCGATTTTGGCTTA -3’, and RPS11 F 5’- CGTGACGAAGATGAAGATGC -3’, R 5’- GCACATTGAATCGCACAGTC -3’. Primers were designed using the NIH Primer-BLAST online tool.

Whole muscle protein extraction and quantification. EDL and SOL muscles were ground into a fine powder using a Cell Crusher tissue pulveriser (Cellcrusher, Cork, Ireland) cooled in liquid nitrogen. The powder was added to a sample tube with a pre-determined volume of RIPA buffer (Sigma-Aldrich, St. Louis, MO; 20 μL of RIPA per 1 mg muscle weight), supplemented with a protease and phosphatase inhibitor cocktail (Roche, Laval, Canada). The solution was subsequently homogenized on ice using sonication (Fisher Scientific, Toronto) at 50% power for 5 X 2 sec, with 30 sec in-between each bout. Samples were spun, and the resulting supernates were collected. The bicinchoninic assay (BCA; Thermofisher Scientific, BioTek, Toronto) was performed in order to determine protein concentrations.

Skeletal muscle cell fractionation. Nuclear, and cytosolic fractions were isolated from SED, 0PE, and 3PE GAST muscles according to procedures described previously ([40]), with modifications. Briefly, muscles were initially ground to a fine powder similar to the description above. 500 μL of STM buffer was added

to each sample tube and the solution was then homogenized on ice using sonication (Fisher Scientific, Toronto) at 100% power for 10 X 2 sec, with 30 sec in-between each bout. An additional 500 μL of STM buffer was added before the samples were spun. After centrifuging at 800 *g* for 15 min the supernatant (S_0) and pellet (P_0) were separated. The pellet (P_0) was resuspended in 1,000 μL of STM buffer, vortexed for 15 seconds and then centrifuged at 500 *g* for 15 min. The nuclear pellet (P_1) was then suspended in 400 μL of STM buffer and spun at 100 *g* for an additional 15 min while the supernatant (S_1) was discarded. Once again the sample was separated and the pellet (P_5) was kept. 400 μL of NET buffer was added to the tube before vortexing and then placed on ice for 30 min. The sample was then sonicated at 100% power for 10 X 2 sec with 30 sec between bouts. The sample was spun one last time at 9,000 *g* for 30 minutes before being separated. The supernatant was kept (S_6) and labelled as the nuclear fraction. The supernatant (S_0) was spun for an additional 10 min at 800 *g* before being separated. The resulting supernatant (S_2) was kept and centrifuged again at 11,000 *g* for another 10 min before additional separation. The supernatant (S_3) was labelled as the cystolic fraction and placed in acetone for 1 hour at -20 °C with a 1:1 ratio of sample to acetone. It was then spun at 12,000 *g* for 5 min before the supernatant was removed and the remaining pellet (P_7) was suspended in 200 μL of STM buffer. Protein concentrations of the cellular fractions were determined with the BCA assay.

Western blot analysis. 20 μg of protein was loaded into each lane of 10-12.5% polyacrylamide gels and subjected to SDS-PAGE, before being transferred to nitrocellulose membranes. After transfer, Ponceau S solution (Sigma, Darmstadt, Germany) was used to verify equal loading across all lanes ([41]). Ponceau solution was washed off with Tris-buffered saline with 1% Tween-20 (TBS-T). Membranes were then blocked with 5% milk for 60 min before being washed 5 X 3 min with TBS-T. Primary antibody dilutions were prepared in 5% milk or bovine serum albumin (BSA) according to the manufacturer's recommendations. The following antibodies were employed: PRMT1 (07-404, EMD Millipore), CARM1 (A300-421A, Bethyl Laboratories), PRMT5 (07-405, EMD Millipore), MMA (8015, Cell Signal), ADMA (13522, Cell Signal), SDMA (13222, Cell Signal), PGC-1α (AB3242, EMD Millipore), AMPK (2535S, Cell Signaling), phosphorylated AMPK (2535S, Cell Signaling), histone H2B (8135, Cell Signalling), GAPDH (ab9483, Abcam), histone 4 arginine 3 (H4R3; 39705, Active Motif), H3R17 (ab8284), H3R8 (ab130740, Abcam), H3 (ab1791), and H4 (ab10158, Abcam). Primary antibodies were applied overnight at 4 °C with gentle shaking and washed off the following morning with 5 X 3 min washes in TBS-T. Appropriate horseradish peroxidase linked secondary antibodies were applied for 2 hrs at room temperature followed by 5 X 3 min washes in TBS-T. Finally, enhanced chemiluminescence substrate (Bio-rad, Berkeley, California) was applied in order to detect target proteins. Images were captured with Alpha Innotech imaging

equipment and ImageJ (NIH) was employed for densitometry.

Statistical Analyses. Differences between group means were evaluated using the ANOVA or Student's t-test analyses, as appropriate. Statistical tests were performed on the -fold differences displayed in the graphical summaries. Statistical differences were considered significant if $p < 0.05$. Data in graphical summaries are means ± SEMs.

Results

Acute exercise evokes intracellular conditions indicative of skeletal muscle remodelling. In order to investigate PRMT expression and function in skeletal muscle during conditions of phenotypic plasticity, we employed a single bout of exercise to elicit an intracellular milieu required for this remodelling process. The SED group acted as a non-stimulated control, while the 0PE group provided an impression of the intracellular environment immediately upon the cessation of exercise. The 3PE mice represented the effects that a recovery period has within the contracting muscle. We first examined the activation of AMPK, as this enzyme is a critical regulator of both acute and chronic skeletal muscle plasticity ([10]), particularly in response to exercise ([8]). Our data demonstrate that phosphorylated AMPK (p-AMPK) content was significantly higher in the EDL (+105%) and SOL muscles (+150%) of the 0PE group as compared to the SED mice, before returning to basal levels at 3PE. (Fig. 1A, C). p-AMPK exhibited a fiber type-specific expression pattern, with the activated enzyme being more highly expressed (+75%;

$p < 0.05$) in the slow, oxidative SOL muscle compared to the faster, more glycolytic EDL muscle. Within each muscle, total AMPK content was similar across the SED, 0PE, and 3PE groups (Fig. 1A, D). In contrast to the levels of p-AMPK, total AMPK content was 35% significantly lower ($p < 0.05$) in the SOL muscle relative to the EDL muscle. AMPK activation status was then calculated as the ratio of the active, phosphorylated form of the enzyme relative to the total content of the protein. AMPK activation status (Fig. 1A, E) reflected the levels of p-AMPK. Active AMPK was ~1.9-2.6-fold higher ($p < 0.05$) immediately post- exercise in the EDL and SOL muscles, and returned to baseline levels with recovery.

Next, we analyzed mRNA levels of PGC-1α and observed a 10-13-fold increase ($p < 0.05$) in both the EDL and SOL muscles following exercise (Fig. 1F). There is some evidence for augmented protein content of the transcriptional coactivator PGC-1α during the acute exercise recovery period ([38]). Our results from EDL and SOL muscles demonstrate that PGC-1α expression was similar across experimental groups within each muscle type (Fig. 1A, G). However, similar to previous studies ([38,42]), PGC-1α levels were significantly higher (+75%) in the SOL versus EDL muscles. In an effort to further characterize the acute exercise response, we separated GAST myocytes into nuclear and cytosolic fractions, as it has been demonstrated that numerous molecules translocate between compartments during and after exercise ([3,43]). The purity of our nuclear and cytosolic samples was confirmed by probing for H2B and GAPDH protein contents, respectively (Fig.

1B). Nuclear PGC-1α expression, which is indicative of its activity ([3,44]), was increased by 1.6-fold ($p < 0.05$) between the SED and 3PE groups (Fig. 1B, H). Cytosolic PGC-1α expression was also found to be significantly higher (+110%) compared to nuclear expression at rest, but contrary to the nuclear samples, acute exercise had no effect on cytosolic PGC-1α content (Fig. 1B, H).

PRMT gene expression in skeletal muscle. We sought to begin our characterization of skeletal muscle PRMT expression and function by measuring PRMT content and localization. Our data indicate that neither acute exercise nor recovery had any effect on PRMT1, CARM1, or PRMT5 transcript levels, or the whole muscle expression of these enzymes (Fig. 2A-G). However, unique fiber type-specificities of PRMT expression were observed. CARM1 mRNA levels were significantly lower in the slow, oxidative SOL muscle, as compared to the faster, more glycolytic EDL muscle (Fig. 2C). PRMT1 and PRMT5 protein content was 1.4-1.80-fold higher ($p < 0.05$) in the SOL muscle versus the EDL muscle (Fig. 2E, G).

In the GAST muscle, we observed a significantly higher level of PRMT expression in the cytosol, as compared to the myonuclei (Fig. 3A-D). A 50% increase ($p < 0.05$) in nuclear PRMT1 abundance was observed at 0PE compared to SED (Fig. 3A, B) followed by a return to resting condition levels at 3PE. In contrast, the nuclear or cytosolic abundance of CARM1 or PRMT5 was unchanged in response to exercise (Fig. 3A, C, D).

Skeletal muscle PRMT activity. We utilized complementary approaches to examine PRMT function in skeletal muscle during the acute response to contractile activity. First, we assessed the content of skeletal muscle MMA, ADMA, and SDMA, as these marks are indicative of PRMT, type I PRMT, and type II PRMT activities, respectively ([11]). Since PRMT1 and CARM1 account for the vast majority of type I PRMT activity, and PRMT5 dominates overall type II activity, this analysis provides some index of distinct PRMT1, CARM1, and PRMT5 function in skeletal muscle. Within each muscle type, the content of MMA, ADMA, and SDMA were similar between SED, 0PE, and 3PE groups (Fig. 4A-C). Once again, a fiber type-specificity was observed, as methylarginine content was significantly higher (1.4-1.9-fold) in the SOL muscle, as compared to the EDL muscle.

Lastly, we investigated the methylation of specific PRMT targets. It is accepted that histone 4 arginine 3 bearing the ADMA modification (H4R3-ADMA), H3R17-ADMA, and H3R8-SDMA have been marked exclusively by PRMT1, CARM1, and PRMT5, respectively ([20]). Our data demonstrate an increase in H4R3 levels at 3PE, as compared to 0PE and SED (Fig. 5A, B). H4 content was similar across experimental groups (Fig. 5A, C). H4R3 methylation status, calculated as the ratio of H4R3 content relative to total H4 levels, was significantly higher in the 0PE and 3PE groups (60-90%), versus the SED group (Fig 5D). The H3R17 and H3R8 marks increased with 2-3-fold ($p < 0.05$) in the 3PE animals, as

compared to the SED group (Fig. 5A, E, F). Similar to H4 levels, skeletal muscle H3 content was similar between SED, 0PE, and 3PE animals (Fig. 5A, G). H3R17 and H3R8 methylation status was significantly higher (~70-110%) in the 3PE mice relative to the SED (Fig. 5H, I).

Discussion

We investigated skeletal muscle PRMT biology within the intracellular environment required for exercise-induced muscle plasticity. Our data demonstrate that PRMT gene expression and global enzyme activity are muscle-specific, generally being higher in slow, oxidative muscle, as compared to faster, more glycolytic fibers. Despite the activation of canonical exercise-induced signalling involving AMPK and PGC-1α, PRMT expression and activity at the whole muscle level were unchanged in response to an acute stimulus for muscle plasticity. However, subcellular analyses revealed the exercise-evoked myonuclear translocation of PRMT1, as well as elevations in targeted CARM1, PRMT1, and -5 methyltransferase activities as indicated by increased histone methylation status. Collectively, these data suggest that the muscle-specificity of PRMT expression may be important for the determination and/or maintenance of disparate fiber type characteristics in skeletal muscle. Moreover, distinct PRMT cellular localization and methyltransferase activity may be key signals that contribute to skeletal muscle phenotypic plasticity.

PRMT gene expression and activity are muscle-specific. We began our characterization of PRMT biology in skeletal muscle by examining enzyme content and activity in muscles of differing fiber type composition. We employed the SOL and EDL muscles as examples of slow oxidative, and fast glycolytic tissues, respectively. Indeed, in mice, the SOL muscle is comprised of 31% type I fibers, 5% type IIA, 12% type IIX, and 3% type IIB, while the EDL muscle is 0% I, 19% IIA, 22% IIX, and 47% IIB ([45]). Our data clearly demonstrate a muscle-specific expression profile for PRMT1, CARM1, and PRMT5. Despite similarities in PRMT1 and PRMT5 transcript levels between EDL and SOL muscles, enzyme protein content was higher in the slower, more oxidative SOL muscle. Furthermore, CARM1 transcript levels were lower in the SOL versus the EDL muscles, while CARM1 protein content was similar between these tissues. Differences in mRNA stability or translational control between muscle types may partly explain the incongruence between PRMT transcript and protein levels, as well as the muscle-specific pattern of expression. These two important steps in the gene expression pathway are uniquely regulated between fast, glycolytic versus slower, more oxidative muscles ([46–48]). Consistent with our data, recent work has also revealed unique muscle-specific expression of PRMTs. For example, PRMT1-6 transcript levels exhibit enzyme-specific expression patterns in murine skeletal muscle of varied fiber composition, with CARM1, PRMT1, and PRMT5 being among the most abundant depending on the muscle studied ([32]). Thus, confirming and

extending earlier work, our data indicate that PRMT1, CARM1, and PRMT5 transcript and protein levels exhibit muscle-specific expression patterns. Future studies investigating muscle fiber type-specific gene expression of PRMTs are warranted in order to extend our knowledge of PRMT biology at the myocellular level.

Cellular MMA, ADMA, and SDMA content are established markers of PRMT, type I PRMT, and type II PRMT activities, respectively ([30,49,50]). In the current study, we investigated the presence of these methylarginine marks in whole muscle homogenates in an effort to understand PRMT function in skeletal muscle in vivo. As expected, PRMT activity generally coincided with PRMT protein levels. Indeed, MMA, ADMA, and SDMA expression levels were significantly more abundant in the slow, oxidative SOL muscle, as compared to its faster, more glycolytic EDL muscle counterpart. This was reflected by the expression pattern of PRMT1 and PRMT5 enzymes, representing the primary type I and type II PRMTs, respectively. All PRMTs generate MMA, however type I PRMTs can additionally deposit the ADMA mark, while type II PRMTs catalyze SDMA production ([15]). More specifically, PRMT1 and PRMT5 account for the majority of ADMA and SDMA synthesis, respectively ([11]). Thus, it is reasonable to assert that in skeletal muscle PRMT1 and PRMT5 activities are dictated, at least in part, by their enzyme abundance. In fact, when averaged across the SED, 0PE, and 3PE groups, the difference in PRMT1 protein content and activity between EDL and SOL muscles

was similar at 1.8-fold, while PRMT5 content and activity were both 1.4-fold higher in the SOL relative to the EDL muscle. Although PRMT1 accounts for ~85% of total methyltransferase activity ([51]), this observation was made in cultured RAT1 fibroblast cells, as well as in mouse liver. Additional work is therefore clearly necessary to determine whether these findings hold true in skeletal muscle.

Numerous studies over the previous 10-15 years, including one in muscle (Chen 2002), have revealed very convincingly that PRMTs regulate the activity of powerful phenotype-bending proteins, such as MEF2, PGC-1α, p53, E2F1, and RIP140 ([23,52–56,27]). For example, Chen and colleagues demonstrated that CARM1 coactivates MEF2-mediated transcription in skeletal muscle cells during myogenesis ([27]). MEF2 is accepted as a master regulator of muscle phenotype determination, maintenance, and plasticity ([3,57]). Notably, PGC-1α, another master determinant of muscle characteristics, is governed by PRMT1-medtiated methylation in CV-1 and COS7 cells ([23]). Despite the fact that most of the investigations cited above were not conducted in skeletal muscle, the data strongly support the hypothesis that PRMTs have the potential to mediate muscle phenotype via similar mechanisms. Nonetheless, our data demonstrate that PRMT expression and function are unique across muscles of differing fiber type composition, which by influencing other potent transcriptional regulators, may play a role in the determination and/or maintenance of disparate fiber type characteristics in skeletal muscle.

PRMT gene expression, localization, and activity are selectively altered during acute skeletal muscle plasticity. We employed a single bout of exercise in order to contextualize PRMT biology within upstream signalling and gene expression cascades that govern skeletal muscle plasticity. We first ensured that our treadmill running protocol was indeed eliciting an intracellular milieu associated with skeletal muscle remodelling. In line with previous studies, a single bout of exercise elevated the activation status of AMPK, induced PGC-1α myonuclear translocation, as well as augmented the mRNA expression of the coactivator ([38,39,58–62]). We therefore capitalized on these findings in order to investigate PRMT expression, localization, and activity during these acute conditions of skeletal muscle plasticity. The data reveal that PRMT transcript and protein levels were unaffected by exercise or recovery. These findings contrast an earlier investigation demonstrating that PRMT1 and CARM1 transcript levels in the muscles of healthy and dystrophic mice increased three hours following a single bout of treadmill running ([31]). Differences in exercise mode (e.g., grade versus downhill running), intensity (e.g., fixed time and speed, versus progressive workload), and/or muscles analyzed (e.g., EDL and SOL, versus TA) may all, or in part, account for the disparate results in PRMT expression between studies. We also observed in the current study that whole muscle MMA, ADMA, and SDMA levels, indicative of global PRMT function, as well as type I and type II PRMT activities, respectively, were unchanged in response to running. Collectively, the absence of any exercise-

evoked alterations in PRMT expression and function at the whole muscle level does not preclude the possibility that changes in, for example, PRMT cellular localization and specific methyltransferase activities did in fact accompany the induction of exercise-responsive signalling. We employed higher resolution analyses, including subcellular fractionation and histone-specific methylation assays, to address this question.

In response to the acute stimulus for muscle remodelling, PRMT1 translocated to myonuclei, while CARM1 and PRMT5 remained concentrated in the cytosolic compartment. Others have noted dynamic PRMT1 cellular localization in skeletal muscle. Iwasaki and Yada (2007) observed that insulin stimulation of L6 myoblasts resulted in PRMT1 accumulation at the sarcolemma relative to its cytosolic and myonuclear occupancy. In addition, recent work from our laboratory demonstrated that CARM1, PRMT1, and -5 display unique patterns of subcellular accumulation throughout a time course of neurogenic muscle disuse ([63]). The cellular localization of PRMTs is subject to multiple levels of regulation. For example, alternative splicing of PRMT1 pre-mRNA can produce several protein isoforms that display distinct subcellular localizations ([64]). Moreover, posttranslational modification of CARM1, such as phosphorylation at Ser217, results in the cytosolic translocation of the methyltransferase ([65]). To our knowledge however, there are no reports examining the effects of posttranslational modification on PRMTs in skeletal muscle. Acute exercise is a potent stimulus for

protein modifications in muscle, such as the phosphorylation of AMPK or the deacetylation of PGC-1α, which in the latter case, results in its myonuclear and mitochondrial accumulation ([3,38]). Elucidating the modifications to PRMTs, if any, during exercise will advance our understanding of the localization and function of these enzymes in skeletal muscle.

It is interesting to note that the exercise-induced myonuclear accumulation of PRMT1 occurred prior to the nuclear translocation of PGC-1α. Previous work linking these two molecules indicate that, under the chronic muscle remodelling conditions elicited by denervation-evoked inactivity, PRMT1 and PGC-1α exist in a complex that can be co-immunoprecipitated ([63]). Furthermore, PRMT1-mediated methylation of PGC-1α potentiates its transcriptional coactivator activity in CV-1 and COS7 cells ([23]). Arginine methylation of PGC-1α occurs in the E region ([23]), which contains a subnuclear localization signal ([66]) that could serve to effectively target the distribution of the coactivator within the organelle. It is therefore reasonable to speculate that PRMT1s myonuclear anticipation of PGC-1α after exercise contributes to a local environment that is permissive to its transcriptional coactivator function. Further studies exploring this possibility would shed light on the emerging relationship between these two powerful phenotype-modifying molecules.

In contrast to our data on whole muscle MMA, ADMA, and SDMA levels, acute muscle remodelling was associated with the increased methylation status of

myonuclear proteins targeted by PRMT1, CARM1, and PRMT5. These results are not surprising since the unique responses of individual PRMTs to exercise likely differ. CARM1, PRMT1, and -5 specifically and exclusively target H3R17, H4R3, and H3R8 with the ADMA (H3R17, H4R3) and SDMA (H3R8) marks, respectively ([15,16,67]). The exercise-induced increase in targeted PRMT activity is likely due, in part, to alterations in PRMT localization and/or arginine methyltransferase functions. Acute physical activity initiated the translocation of PRMT1 into myonuclei, which increases its proximity to H4 within the organelle. There is evidence to suggest that the major site of PRMT1 activity is within nuclei, as inhibition of methylation leads to a significant increase in the nuclear accumulation of the enzyme ([68]). Distinct from PRMT1, the subcellular distribution of CARM1 and PRMT5 proteins was unchanged immediately after physical activity, as well as 3 hours post-exercise. However, we observed an exercise-induced increase in H3R17 and H3R8 methylation status, indicative of enhanced CARM1 and PRMT5 myonuclear activity, respectively. As noted above, PRMTs display basal activity without requiring processing, however PRMT biology can be affected by posttranslational modification, and CARM1, PRMT5, as well as PRMT1 arginine methyltransferase activities are affected by phosphorylation ([15,17,69,70]). In fact, PRMTs exhibit autoregulatory activity via arginine methylation, which can fine-tune enzyme function ([15,69]). Earlier evidence indicates that exercise results in post-translational modifications of histones, which in turn mediate

alterations in skeletal muscle gene expression ([71,72]). Our data indicate an association between exercise-induced signalling and PRMT-mediated histone arginine modification, which suggest that PRMT methyltransferase activity in myonuclei may play an important role in the adaptive response to physical activity.

It is important to note that, despite alterations in PRMT localization and activity in the myonuclear compartment in response to exercise, the vast majority of PRMT protein was found in the cytosolic fraction. These data corroborate previous investigations demonstrating that PRMT content is significantly greater in the cytosol, as compared to within nuclei ([73,74]). As such, future studies should also focus on determining PRMT expression and function in the cytosolic compartment. For example, recent studies have demonstrated that PRMTs may play a role in altering mitochondrial homeostasis by methylating various protein targets associated with organelle function, such as BCL-2 antagonist of cell death, malate dehydrogenase 1, and others ([75–77]). Investigating the potential links between PRMTs and mitochondria within muscle is a worthy endeavour since the function of this organelle is central to numerous critical aspects of skeletal muscle biology.

In conclusion, our data demonstrate that PRMT1, CARM1, and PRMT5 exhibit muscle-specific expression and function in mice. In response to a robust stimulus for muscle remodelling, PRMT1 displays myonuclear accumulation prior to the nuclear translocation of PGC-1α, which colocalizes the proteins within the organelle after exercise. Acute physical activity also augmented the targeted

methyltransferase activities of CARM1, PRMT1, and -5 in the myonuclear compartment, suggesting that PRMT-mediated histone arginine methylation is an integral part of the early signals that drive skeletal muscle plasticity. To expand our knowledge of PRMT biology in skeletal muscle, future work is required to explore the mechanisms that control PRMT localization and enzyme activity, including the potential relationship between PRMTs and key muscle phenotype determination and remodelling molecules.

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Declaration of Academic Achievement

Tiffany L vanLieshout was the principal contributor. Derek W Stouth assisted with conceiving and designing the study. Tania Tajik assisted with Western blotting. Vladimir Ljubicic assisted with conceiving and designing the study, as well as with writing the manuscript.

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Energy Metabolism and Homeostasis in Caenorhabditis elegans. *Mol. Cell. Biol.* **37,** e00504-16 (2017).

Figure Legends

Figure 1: Acute exercise stimulates signalling for muscle remodelling. *A:* Representative Western blots for phosphorylated AMP-activated protein kinase (p-AMPK), total AMPK, and peroxisome proliferator-activated receptor γ coactivator-1α (PGC-1α) in the extensor digitorum longus (EDL) and soleus (SOL) muscles harvested from mice that were sedentary (SED), immediately post-exercise (0PE), or 3 hours after exercise (3PE). The ponceau stain demonstrates equal loading between sample lanes. *B:* Typical Western blots for PGC-1α, glyceraldehyde 3-phosphate dehydrogenase (GAPDH), and histone 2B (H2B) in nuclear (N) and cytosolic (C) cellular compartments from gastrocnemius (GAST) muscles. Dashed lines indicate the splicing of non-adjacent lines from the same image. Graphical summaries of p-AMPK content (*C*), total AMPK protein content (*D*), AMPK activation status, calculated as the level of p-AMPK relative to AMPK content *(E*), PGC-1α mRNA levels (*F)*, and PGC-1α protein content (*G*) in EDL and SOL muscles from the three experimental groups. Data are displayed as relative to SED EDL levels. *, $p < 0.05$ vs. SED within each muscle; †, $p < 0.05$ vs. 0PE within each muscle; #, $p < 0.05$ vs. EDL. $n = 5 – 12$. *H:* Graphical summary of PGC-1α nuclear and cytosolic content in GAST muscles from SED, 0PE, and 3PE groups. Data are displayed as relative to SED nuclear levels. *, $p < 0.05$ vs. SED within each muscle; #, $p < 0.05$ vs nuclear. $n = 6 – 9$.

Figure 2: PRMT gene expression in skeletal muscle. *A:* Typical Western blots for protein arginine methyltransferase 1 (PRMT1), co-activator-associated arginine methyltransferase 1 (CARM1), and PRMT5 in the EDL and SOL muscles from SED, 0PE, and 3PE groups. The ponceau stain demonstrates equal loading. Graphical summaries of PRMT1 (*B*), CARM1 (*C*), and PRMT5 (*D*) transcript levels, as well as PRMT1 (*E*), CARM1 (*F*), and PRMT5 (*G*) protein content in EDL and SOL muscles from the three experimental groups. Data are displayed as relative to SED EDL levels. #, $p < 0.05$ vs. EDL. $n = 4 – 12$.

Figure 3: PRMT localization during conditions of acute muscle plasticity. *A:* Representative Western blots of PRMT1 (short and long exposure), CARM1, and PRMT5 (short and long exposure) levels in nuclear and cytosolic compartments of GAST muscles from the three experimental groups. The ponceau stain demonstrates equal loading. Graphical summaries of PRMT1 (*B*), CARM1 (*C*), and PRMT5 (*D*) protein content in GAST muscle nuclear and cytosolic fractions. Data are displayed as relative to SED nuclear fraction levels. *, $p < 0.05$ vs. SED within each muscle; †, $p < 0.05$ vs. 0PE within each muscle; #, $p < 0.05$ vs nuclear. $n = 9 – 12$.

Figure 4: Global PRMT activity in skeletal muscle. Typical immunoblots of monomethylarginine (MMA; *A*), asymmetric dimethylarginine (ADMA; *B*), and

symmetric dimethylarginine (SDMA; *C*) marks in EDL and SOL muscles. Approximate molecular weights (MW) in kilodaltons (kDa) are indicated on the left of each image. On the right of each panel are graphical summaries of the muscle MMA, ADMA, and SDMA content from the SED, 0PE, and 3PE groups. Data are displayed as relative to SED EDL levels. #, $p < 0.05$ vs. EDL. $n = 7 – 8$.

Figure 5: PRMT-specific function during exercise-induced skeletal muscle plasticity. *A:* Representative Western blots for the ADMA mark on histone 4 arginine 3 (H4R3), H4, the ADMA-marked H3R17 (H3R17), the SDMA modification on H3R8 (H3R8), and H3, in enriched GAST myonuclear samples. The ponceau stain demonstrates equal loading. Graphical summaries of the levels of H4R3 (*B*), H4 (*C*), H4R3 methylation status, calculated as the H4R3 content relative to the total amount of H4 (*D*), H3R17 (*E*), H3R8 (*F*), H3 (*G*), H3R17 methylation status (*H*), and H3R8 methylation status (*I*) in the three experimental groups. Data are displayed relative to SED levels. $n = 7 – 8$.

Figure 1

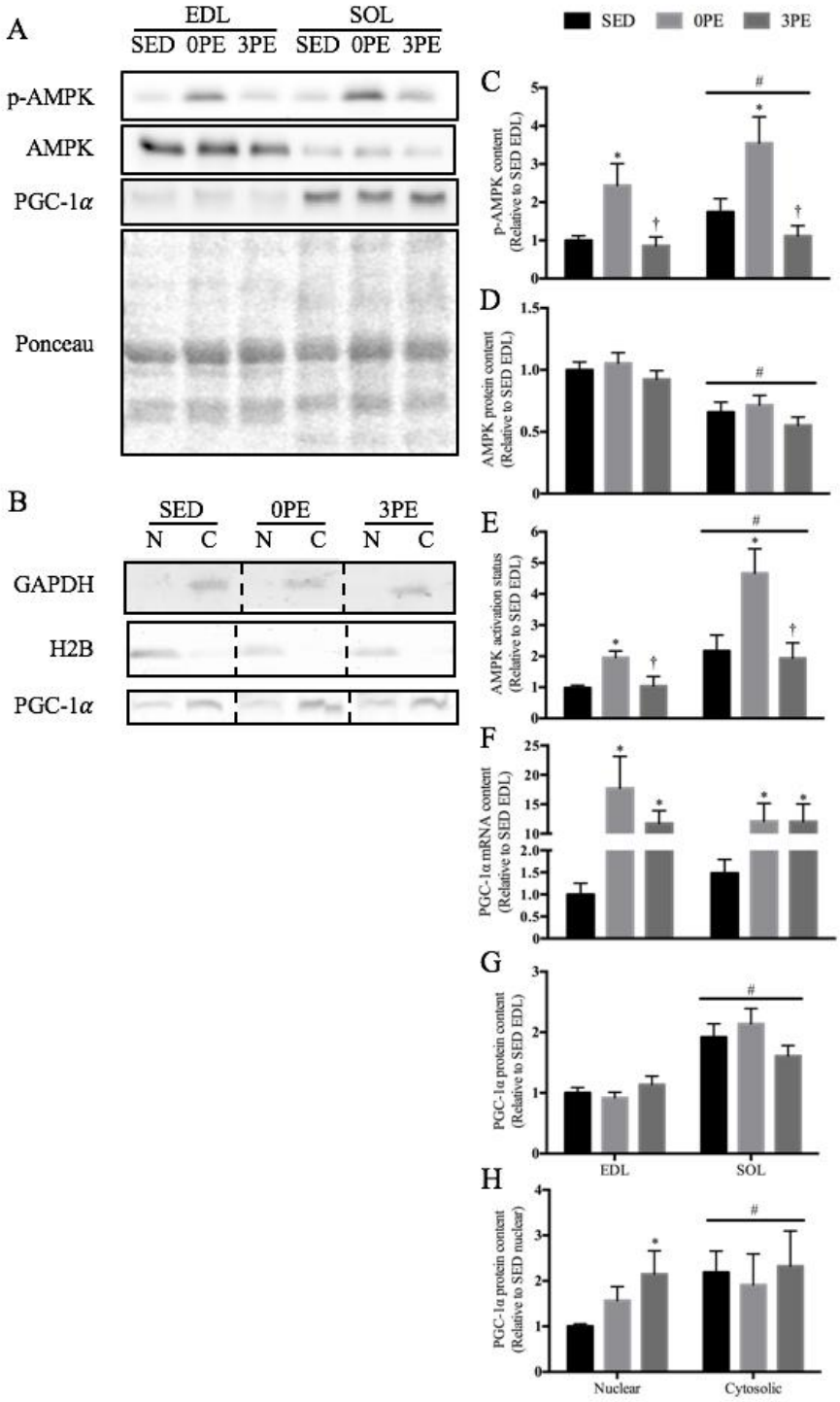

A
EDL
SOL
SED 0PE 3PE SED 0PE 3PE
p-AMPK
AMPK
PGC-1α
Ponceau
B
SED
0PE
3PE
N C N C N C
GAPDH
H2B
PGC-1α
SED 0PE 3PE
C
p-AMPK content (Relative to SED EDL)
D
AMPK protein content (Relative to SED EDL)
E
AMPK activation status (Relative to SED EDL)
F
PGC-1α mRNA content (Relative to SED EDL)
G
PGC-1α protein content (Relative to SED EDL)
EDL
SOL
H
PGC-1α protein content (Relative to SED nuclear)
Nuclear
Cytosolic

Figure 2

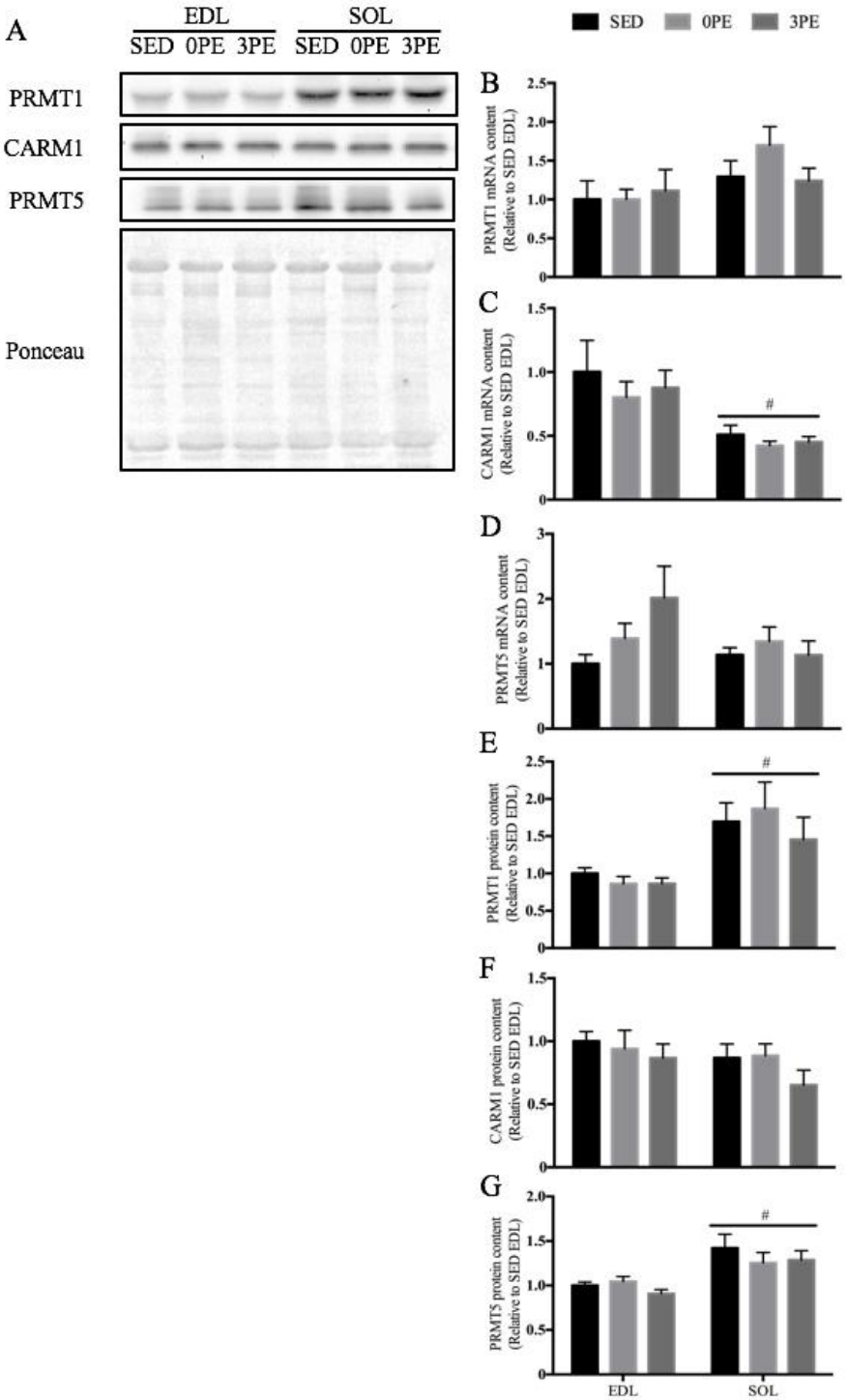
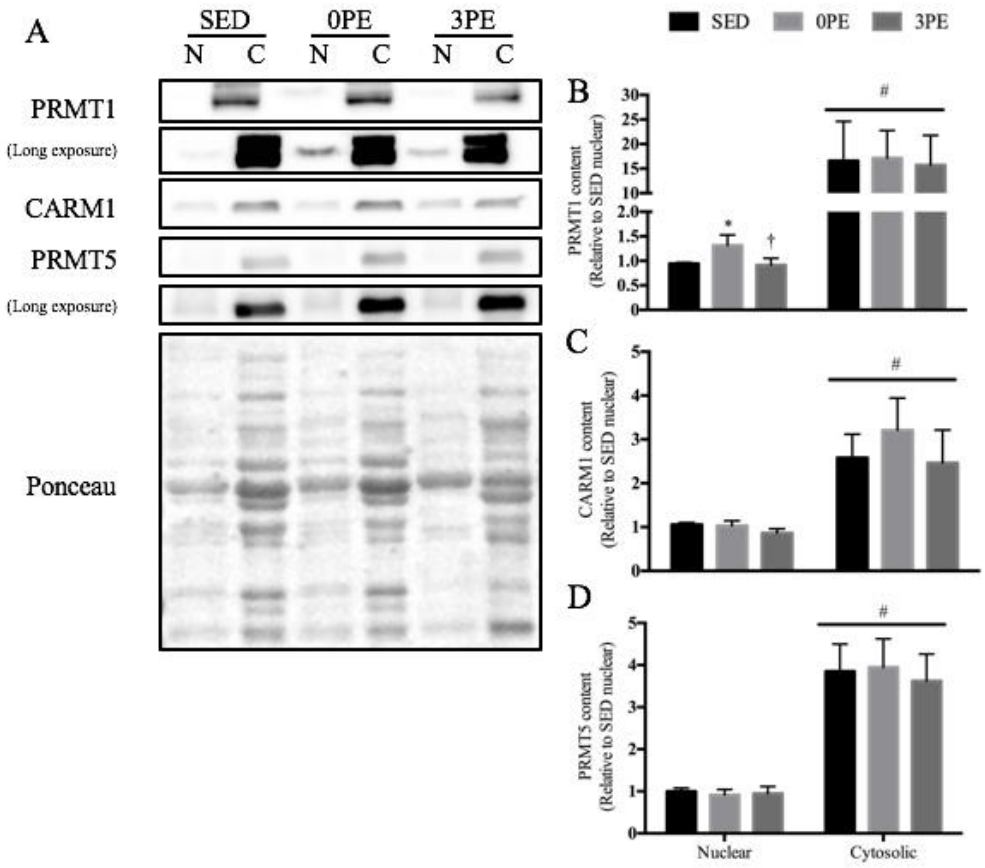

A
EDL
SOL
SED 0PE 3PE SED 0PE 3PE
PRMT1
CARM1
PRMT5
Ponceau
SED
0PE
3PE
B
PRMT1 mRNA content (Relative to SED EDL)
C
CARM1 mRNA content (Relative to SED EDL)
#
D
PRMT5 mRNA content (Relative to SED EDL)
E
PRMT1 protein content (Relative to SED EDL)
#
F
CARM1 protein content (Relative to SED EDL)
G
PRMT5 protein content (Relative to SED EDL)
#
EDL
SOL

Figure 3


A
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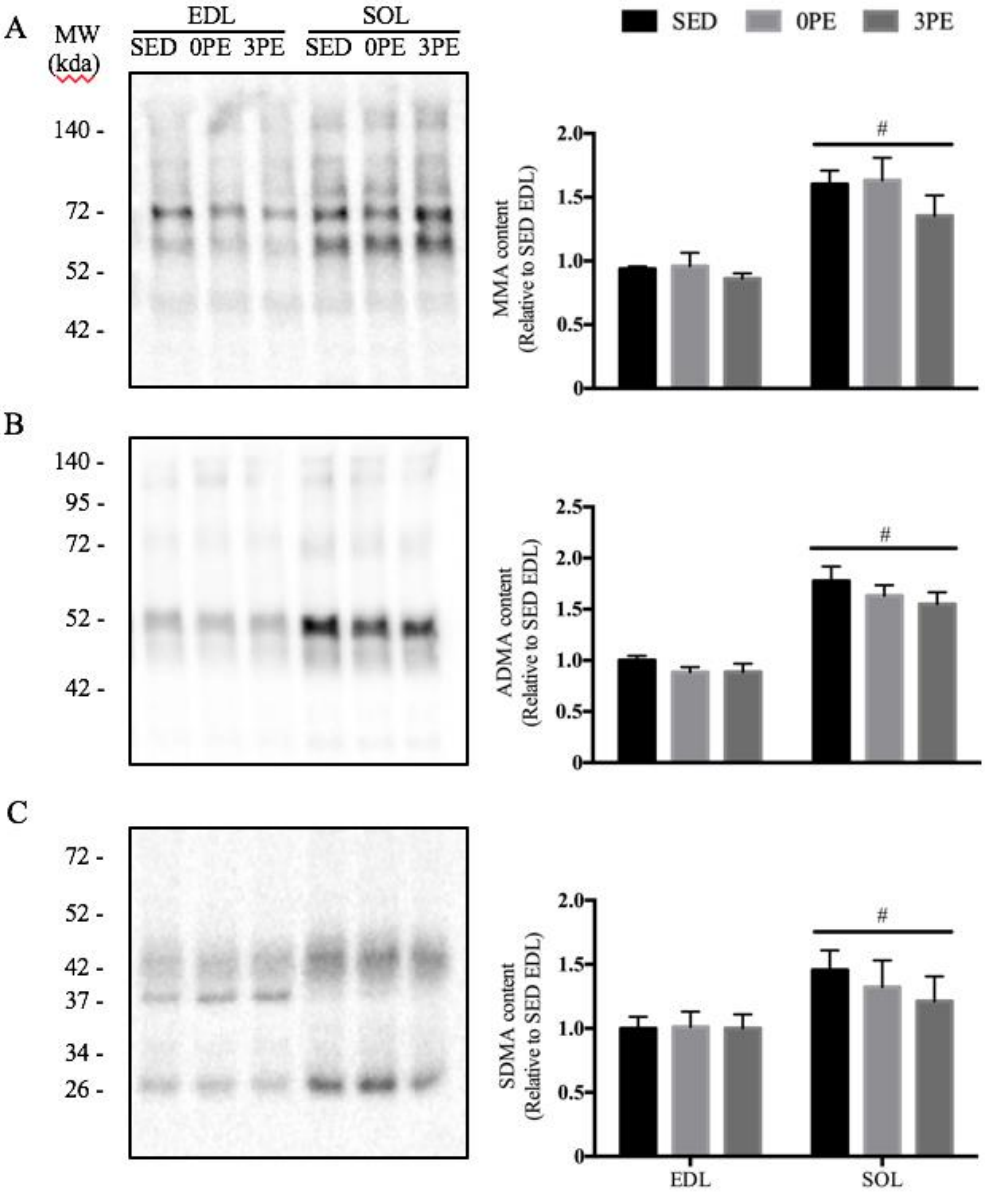
PRMT1
(Long exposure)
CARM1
PRMT5
(Long exposure)
Ponceau
SED
0PE
3PE
B
PRMT1 content
(Relative to SED nuclear)
C
CARM1 content
(Relative to SED nuclear)
D
PRMT5 content
(Relative to SED nuclear)
Nuclear
Cytosolic

Figure 4


A
MW (kda)
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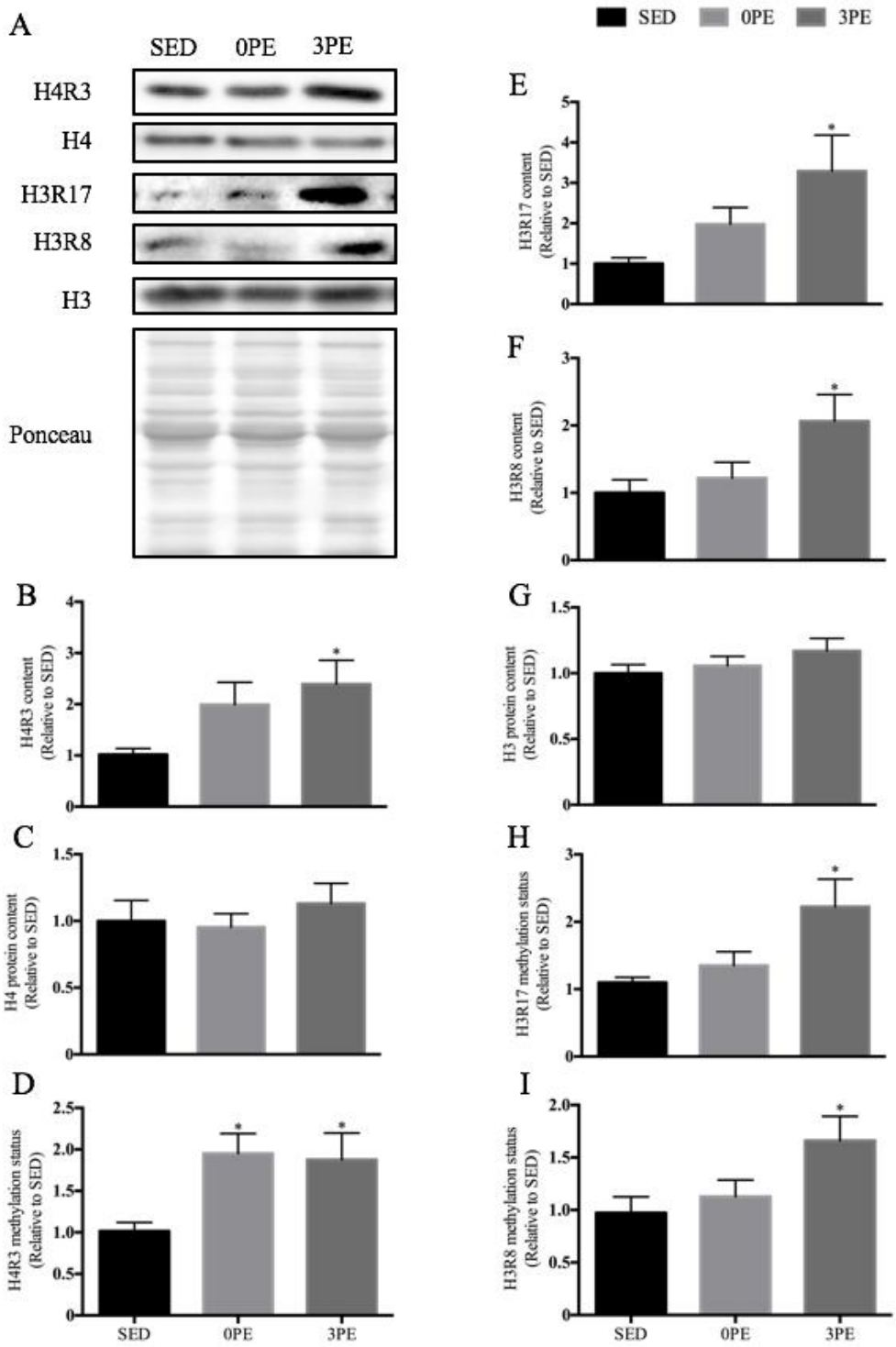
SOL
SED 0PE 3PE SED 0PE 3PE
140 -
72 -
52 -
42 -
SED
0PE
3PE
MMA content (Relative to SED EDL)
#
B
140 -
95 -
72 -
52 -
42 -
ADMA content (Relative to SED EDL)
C
72 -
52 -
42 -
37 -
34 -
26 -
SDMA content (Relative to SED EDL)
EDL
SOL

Figure 5


A
SED
0PE
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H4R3
H4
H3R17
H3R8
H3
Ponceau
B
H4R3 content (Relative to SED)
C
H4 protein content (Relative to SED)
D
H4R3 methylation status (Relative to SED)
SED
0PE
3PE
E
H3R17 content (Relative to SED)
F
H3R8 content (Relative to SED)
G
H3 protein content (Relative to SED)
H
H3R17 methylation status (Relative to SED)
I
H3R8 methylation status (Relative to SED)
SED
0PE
3PE