Role of SERCA Pump in Muscle Thermogenesis and Metabolism

Muthu Periasamy,[*1] *Santosh Kumar Maurya,*[1] *Sanjaya Kumar Sahoo,*[1] *Sushant Singh,*[1] *Felipe C. G. Reis,*[1] *and Naresh Chandra Bal*[2]

ABSTRACT

In muscle cells, the sarcoplasmic reticulum (SR) not only acts as a Ca^{2+} store, but also regulates the contractile characteristics of the muscle. Ca^{2+} release from the SR is the primary mechanism for activating muscle contraction and reuptake of Ca^{2+} by the sarcoplasmic reticulum Ca^{2+} ATPase (SERCA) pump causes muscle relaxation. The SERCA pump isoforms are encoded by three genes, SERCA 1, 2, and 3, which are differentially expressed in muscle and determine SR Ca^{2+} dynamics by affecting the rate and amount of Ca^{2+} uptake, thereby affecting SR store and release of Ca^{2+} in muscle. In muscle, small molecular weight proteins, including Phospholamban (PLB) and Sarcolipin (SLN), also regulate the SERCA pump. Regulation of the SERCA pump by PLB or SLN affects cytosolic Ca^{2+} dynamics and changes in cytosolic Ca^{2+} not only affect contractile function, but also mitochondrial ATP production. Recent studies have shown that alterations in cytosolic Ca^{2+} affects Ca^{2+} entry into mitochondria and ATP production; thus, Ca^{2+} serves as an integrating signal between muscle contraction-dependent energy demand and mitochondrial energy production. In addition, changes in cytosolic Ca^{2+} can affect Ca^{2+} signaling pathways modulating gene expression and muscle growth. An emerging area of research shows that SR Ca^{2+} cycling is also a player in muscle-based nonshivering thermogenesis. Recent data shows that SERCA uncoupling by SLN leads to increased ATP hydrolysis and heat production. Our studies, using genetically altered mouse models of SLN, show that SLN/SERCA interaction plays an important role in muscle thermogenesis and metabolism, which will be discussed here, in great length. © 2017 American Physiological Society. *Compr Physiol* 7:879-890, 2017.

Introduction

Skeletal muscle is the largest organ in the body, representing ~40% of body mass, and participates in many physiological functions of the body (57). Much of the early research has been focused on muscle as a contractile organ which led to the characterization of skeletal muscle fibers as fast glycolytic, fast oxidative, and slow-twitch muscle with different contractile kinetics expressing different sets of myosin isoforms (50,82,104-106). These skeletal muscle fibers also have different metabolic characteristics and have the ability to utilize a large amount of fuel when activated. There is increasing evidence that skeletal muscle is more than contractile machinery; it can also contribute to muscle thermogenesis through both shivering and nonshivering mechanisms (4, 8, 9, 100). Skeletal and cardiac muscle are powerhouses; together they consume a significant amount of energy in the body on a daily basis. Skeletal muscle, for example, consumes nearly 80% of available glucose and can easily switch to utilize fatty acids, ketones, and amino acids; thus, it has enormous flexibility in substrate oxidation (41, 61). Therefore, muscle is also considered to be an important regulator of basal metabolic rate, whole body energy expenditure, and metabolism (134). Muscle can increase its energy expenditure 20- to 30-fold during intense exercise and continued exercise can exhaust all of the fat reserve. Many studies suggest that enhancing energy expenditure in muscle through physical activity could be the most effective strategy for controlling obesity and diabetes, second only to caloric restriction (125). Although increased physical activity through exercise is the most preferred way to increase energy expenditure, it is often difficult to enforce and maintain; therefore, we may need to explore alternate mechanisms that could be targeted to activate energy expenditure in muscle and other organs. In striated muscle, the contractile protein machinery and ion transport by the SR membrane network are the central players in energy expenditure (4, 10, 92). Studies in our laboratory have been focused on understanding the role of SR Ca^{2+} ion transport in muscle metabolism/energy expenditure. The purpose of this review is to highlight the role of SR Ca^{2+} cycling, especially the role of sarcoplasmic reticulum Ca^{2+} ATPase (SERCA) pump activity in muscle nonshivering thermogenesis and metabolism. In this review, we will discuss recent advances in our

[*]Correspondence to mperiasamy@SBPdiscovery.org

[1]Sanford Burnham Prebys Medical Discovery Institute at Lake Nona, Orlando, Florida, USA

[2]School of Biotechnology, KIIT University, Bhubaneswar, Odisha, India

Published online, July 2017 (*comprehensivephysiology.com*)

DOI: 10.1002/cphy.c160030

understanding of the SERCA pump activity by a small protein, namely, Sarcolipin (SLN) and its role in muscle nonshivering thermogenesis and energy metabolism (9).

Sarcoplasmic Reticulum Structure and Function

In striated muscle, the SR is unique and highly specialized; it is an elaborate intracellular membrane network organized as discreet units along the sarcomere (12). The SR membrane network interdigitates with T-tubular systems that originate from the plasma membrane and together, they regulate Ca^{2+} movements during muscle contraction and relaxation (16,44). Unlike cardiac muscle, skeletal muscle has well developed SR and T-tubular systems that depend on motor neurons for excitation-contraction coupling. Direct coupling of the dihydropyridine receptor (DHPR) to the ryanodine receptor (RyR, SR Ca^{2+} release channel) regulates the release of Ca^{2+} from the SR (13). Cardiac and skeletal muscles express unique isoforms of these channels and they show both structural and functional differences. The skeletal muscle DHPR is unique in that it has the ability to physically couple and activate RyR1 in a voltage-dependent manner; whereas the cardiac L-type Ca^{2+} channel communicates indirectly through Ca^{2+} induced Ca^{2+} release (45, 93). Likewise, cardiac and skeletal muscles express different isoforms of luminal SR proteins that include triadin, junctin, and calsequestrin (CASQ) (132). In the resting state of the myofiber, Ca^{2+} concentrations in the cytosol are maintained between 50 and 100 nmol/L. The Ca^{2+} cycle starts with a surface membrane and transverse tubular (T system) depolarization leading to a release of Ca^{2+} from the SR via the ryanodine receptor (RyR) that elevates cytosolic Ca^{2+} locally to ~100-fold higher levels (72,91). SERCA is responsible for transporting Ca^{2+} into the lumen of the SR and is the most abundant protein in the SR (Fig. 1). Both SERCA 1 and 2 isoforms are expressed in skeletal muscle, while cardiac muscle expresses primarily the SERCA 2a isoform. The Ca^{2+} removal is also facilitated by the plasma membrane Ca^{2+} ATPases (PMCAs) and Na^+/Ca^{2+} exchangers (91). In comparison to fast-twitch skeletal muscle, Ca^{2+} handling in slow-twitch muscle promotes slow, sustained contractions as found in postural muscle. The Ca^{2+} uptake activity of SERCA pump is influenced by Phospholamban (PLB) and SLN in both cardiac and skeletal muscle (72, 91, 108).

The Role of SERCA Pump Isoforms in Muscle Physiology

The SERCA pump belongs to the family of P-type ATPases that includes PMCA, Na^+/K^+ ATPase and H^+/K^+ ATPase (21, 118). The SERCA pump is a single polypeptide consisting of 110kDa and is localized in the SR membrane. A notable feature of P-type ATPases is the transfer of terminal phosphate from ATP to an aspartate residue in the catalytic

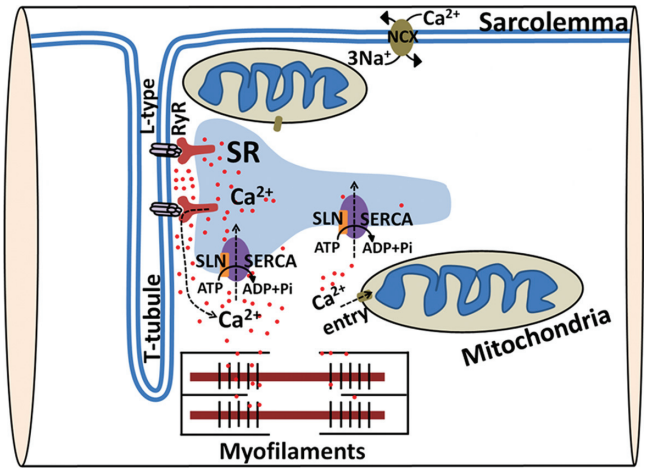

Figure 1 The role of sarcoplasmic reticulum in muscle excitation-contraction coupling. The SR in muscle is a highly specialized organelle and serves as a Ca^{2+} store. Ca^{2+} release and uptake by the SR is primarily responsible for contraction and relaxation of the muscle. The major SR Ca^{2+} cycling proteins include the Ca^{2+} release channel also known as ryanodine receptor (RYR), SERCA pump, Na^+/Ca^{2+} exchanger, and voltage gated L-type Ca^{2+} channel. These proteins regulate Ca^{2+} release and removal during muscle contraction and relaxation (12). The SERCA pump isotype and relative protein content is an important determinant of SR Ca^{2+} load, release, and uptake in fast-twitch (SERCA1a) versus slow-twitch (SERCA2a) muscle. The SR and mitochondria are interdependent and changes in cytosolic Ca^{2+} can also affect mitochondrial energetics.

domain, resulting in a reversible conformational change. P-type ATPases couple the hydrolysis of ATP to the movement of ions across a biological membrane (87, 88, 112, 117, 120-123). The SERCA pump utilizes the energy derived from ATP hydrolysis to transport Ca^{2+} against a Ca^{2+} gradient across SR membranes. The mechanism of the coupling process is such that two Ca^{2+} ions are transported for each molecule of ATP hydrolyzed. During this course of action, a portion of chemical energy is released as heat. Structurally, SERCA is a single polypeptide chain with a transmembrane region (TM) and a large cytoplasmic region, composed of three domains, the nucleotide or ATP binding (N)-domain, the P-domain which gets phosphorylated with the γ-phosphate of ATP and the actuator (A)-domain which coordinates de-phosphorylation (80, 118). These TM regions consist of 10 α-helices (M1-M10) of varying lengths and are in association with highly mobile cytosolic domains. The SERCA isoforms are encoded by three different genes; SERCA 1, SERCA 2, and SERCA 3 (Table 1) (91). SERCA 1 is expressed primarily in fast-twitch skeletal muscle and is alternatively spliced to encode SERCA 1a (994aa, adult) and 1b (1011aa, neonatal). SERCA 2 encodes SERCA 2a (997aa), which is expressed predominantly in cardiac and slow-twitch skeletal muscle and SERCA 2b (1042aa), which is expressed in all tissues at low levels, including muscle and nonmuscle cells. SERCA 3 isoforms are expressed in several nonmuscle tissues but appear to be a minor form in muscle (91). An important feature associated with different SERCA isoforms is that their primary structure

Table 1 The SERCA Pump Isoforms Are Encoded by SERCA1, 2, and 3 Genes

SERCA isoforms	Cardiac muscle	Skeletal muscle		Nonmuscle cells
		Fast twitch	Slow twitch	
SERCA 1a	None	Yes	None	None
SERCA 1b	None	Yes	None	None
SERCA 2a	Yes	Yes	Yes	None
SERCA 2b	Yes	Yes	Yes	Yes
SERCA 3a	None	None	None	Yes
SERCA 3b	None	None	None	Yes
SERCA 3c	None	None	None	Yes

SERCA1a is the most abundant isoform and expressed in adult fast-twitch skeletal muscle; SERCA1b is expressed in neonatal stages of muscle development. SERCA2a is predominantly expressed in cardiac muscle and as well in neonatal and adult slow-twitch skeletal muscle. SERCA2b is ubiquitous and found in most cell types. SERCA 3a, 3b, and 3c are absent in muscle but expressed in many nonmuscle cells including, lung, kidney, spleen, intestine, pancreas, cerebellum, and platelets (91).

is highly conserved and various domains can be exchanged between SERCA 1 and SERCA 2 isoforms without affecting function. SERCA 1 bears structural homology up to 84% with SERCA 2a and 75% to SERCA 3. Due to this similarity in their native primary structure, all SERCA isoforms are predicated to have the similar transmembrane topologies and tertiary structures. SERCA expression is not only tissue specific, but also undergoes developmental regulation, including switching of isoforms, which represents an important phenotypic change in muscle maturation. SERCA 1a isoform predominates in muscle fibers with faster contractile characteristics whereas SERCA 2a seems to be characteristic of slow and cardiac muscle. Fast-twitch muscle expresses threefold to fourfold higher SERCA level (SERCA1a) compared to slow-twitch muscle that expresses SERCA 2a protein. The SERCA protein level and isotype determines the properties of muscle SR and correlates with muscle contractile speed and relaxation.

Skeletal Muscle Is Also a Heat-Generating Organ

Skeletal muscle has been appreciated as a major site of cold-induced thermogenesis through shivering and nonshivering thermogenesis (15, 28, 65, 109, 127). Shivering, a form of repetitive contraction of muscle is the first line of defense against exposure to acute cold environments that produces large amounts of heat, but at the expense of a lot of energy. Prolonged shivering can lead to muscle fatigue and exhaustion as it primarily relies on glycolysis for ATP supply; it can also severely compromise survival of the animal in the wild habitat (26,30,49). Several studies suggest that shivering cannot continue for a very long time and needs to be replaced through nonshivering thermogenic (NST) mechanisms (7, 48, 115). During prolonged cold adaptation, it has been consistently shown that skeletal muscle also serves as an important site of NST (38,59,114,133). Shivering starts to reduce after 2 days of cold challenge and no visible shivering is observed after 4 days. Predominant use of rodents (mice and rats) as experimental animals has often neglects the role of skeletal muscle in NST, as rodents rely on brown adipose tissue (BAT), a highly specialized organ enriched with mitochondria, as the major site of NST (23,24,39,75,131). This is largely because rodents are endowed with BAT and BAT-based thermogenesis plays a dominant role in temperature homeostasis even during adulthood (23, 24, 39, 75, 131). In large mammals, however, BAT content decreases during development; it becomes a minor component or is often inactive in adult stages especially in large animals including humans (22,25,64,66). Furthermore, it is either absent or inactive in some endotherms, even in certain mammals such as wild boars and domesticated pigs (11, 84). In addition, birds that maintain a higher body temperature than mammals, do not contain BAT altogether and they rely on muscle-based thermogenic mechanisms for their core body temperature (Tc) maintenance.

Heat generation from muscle contractility through shivering and/or exercise is well known (28, 40). However, the precise cellular mechanism skeletal muscle utilizes to heat through NST has been controversial (109, 114). In fact, several articles have cited skeletal muscle as an important site of NST but often cannot distinguish if this was occurring as a result of muscle activity or independent of muscle contraction (33-35). There is experimental evidence that Ca^{2+} cycling and SERCA activity can also contribute to heat production in muscle and support thermogenesis in many endothermic vertebrates (33, 62). This is best known from the studies carried out on the "heater organ": a modified extraocular muscle found in deep sea fish, for example, Tuna. The heater organ is composed of cells that lack the typical myofibrillar lattice but, instead are densely packed with mitochondria and SR networks. The SR of heater organs is organized into tightly packed stacks of vesicles, which optimizes surface area for SERCA expression/localization (17,67,81,85). These SR vesicles express a Ca^{2+} release channel (CRC) that is distributed homogeneously throughout with calsequestrin being abundantly located inside the SR lumen.

The role of SR Ca^{2+} cycling in heat production was also supported by a disease, Malignant Hyperthermia (found in pigs and man), where excessive Ca^{2+} leaks from RyR coupled with chronic SERCA activation leading to pathological heat production (20,56,60,96,97,126). While this is primarily due to a mutation in the RyR1 protein that results in an uncontrolled Ca^{2+} leak from the SR following an exposure to anesthetics, it signifies how incessant Ca^{2+} cycling can lead to abnormal heat production. Many recent studies also suggested that muscle adaptation to cold was often associated

Figure 2 Comparison of SLN, DWORF, MLN, and PLB protein sequences in mouse. SLN is 31 AA long with 7 unique cytosolic residues and a highly conserved C-terminus (RSYQY) across different species including humans and rodents. These proteins share homology only in the transmembrane region but have unique cytosolic and lumenal residues. PLB is the largest, it is 52 AA long, and has an extended cytosolic domain of 30 AA containing phosphorylation sites at Ser16 and Thr17(74). The mechanism of interaction with SERCA has been extensively studied for PLB and SLN but the details regarding DWORF and MLN interaction are not known.

with increased RyR1 and SERCA expression in birds and mammals, thus, implying that SR Ca^{2+} transport can be an important contributor to heat production in muscle; however, the mechanistic basis for the recruitment of SR Ca^{2+}-cycling independent of muscle contraction remained poorly understood. The role of SR Ca^{2+} transport in NST has been strengthened by the identification of SLN, a novel regulator of SERCA as described below (9, 90, 99-100, 103).

Regulation of SERCA Activity by Small Molecular Weight Proteins

The SERCA pump activity in muscle is regulated by small proteins, such as PLB, SLN, more recently identified myoregulin (MLN) and dwarf open reading frame (DWORF) (3, 72, 83, 108) (Fig. 2). These small proteins are differentially expressed in muscle, and regulate SERCA isoforms differently. Data shows that PLB primarily interacts with SERCA2a, whereas SLN regulates both SERCA 1 and 2 isoforms (91). On the other hand, PLB does not bind nor affect SERCA3a activity (116). DWORF can bind to all the three isoforms of SERCA (83). PLB, SLN and MLN share a conserved hydrophobic motif in the TM region rich in Leucine residues that provide interaction surface for binding to SERCA. The importance of this hydrophobic TM region was studied by peptide reconstitution by Afara et al. This study revealed that a uniform hydrophobic peptide can alter the apparent SERCA pump affinity for Ca^{2+}, with a length requirement that is similar to WT PLB (1). The role of PLB as a regulator of cardiac muscle physiology is well established; PLB binding to SERCA decreases the pump affinity for Ca^{2+} but this inhibitory interaction is relieved upon phosphorylation or elevated cytosolic Ca^{2+} concentration. Thus, PLB serves as a force frequency modulator of heart function and is the principle mediator of the β-adrenergic response of the heart (63,70). Many studies using mouse models highlighted the role of PLB in cardiac pathophysiology and numerous excellent reviews have been published on this topic, so it will not be discussed in detail here (72). On the other hand, the physiological relevance of SLN is still an emerging area of research. SLN is a small protein, composed of 31 amino acids, and is primarily expressed in striated muscle; not in smooth muscle or any other cell type (5, 14). Interestingly, SLN expression is developmentally regulated in rodents; it is abundant in fetal and neonatal skeletal muscles but gradually becomes restricted to slow-twitch oxidative muscle fibers, such as those in the soleus and diaphragm muscles, in adult rodents (5). In cardiac muscle, SLN is relatively abundant in the atrial muscle compared to ventricles (5). In comparison to rodents, SLN is expressed several fold higher in both fast and slow-twitch muscles in larger adult mammals, such as rabbits, dogs, and humans. MLN, a newly identified regulator of SERCA, is found to be expressed in fast-twitch skeletal muscle encoded by skeletal muscle-specific RNA annotated as a putative long noncoding RNA. MLN is 46 amino acids long and interacts with SERCA, inhibiting its function (3, 83). Genetic ablation of MLN improves exercise performance. Recently, another 34 amino acid peptide was identified and named DWORF. It is encoded by a putative muscle-specific long noncoding RNA and expressed primarily in the ventricles and soleus of mice. Genetic manipulation studies suggested that DWORF activates SERCA pump and increases muscle contractility in the heart by displacing PLB (83). The detailed mechanism of MLN and DWORF interaction with SERCA is currently under investigation and their physiological roles need to be better defined.

SLN Uncouples SERCA Pump from Ca^{2+} Transport

The mechanism of SLN interaction with SERCA has been investigated both by our group and others (Fig. 3) (9, 73, 102, 103, 108). Using protein cross-linking, we were the first to report that monomeric SLN binds to the transmembrane groove of SERCA in a Ca^{2+}-dependent manner and this finding was subsequently confirmed by SERCA/SLN co-crystals developed by Drs. Nissen and Toyoshima's laboratories (119, 129). Furthermore, we showed that the characteristics of SLN binding to SERCA are quite different from PLB, a well-known inhibitor of SERCA. PLB binds to Ca^{2+} free SERCA states and is out-competed by Ca^{2+}binding; therefore, binding of PLB and Ca^{2+} with SERCA are mutually exclusive (9, 102, 103). On the other hand, SLN can bind to Ca^{2+} bound SERCA states and this can be detected even at

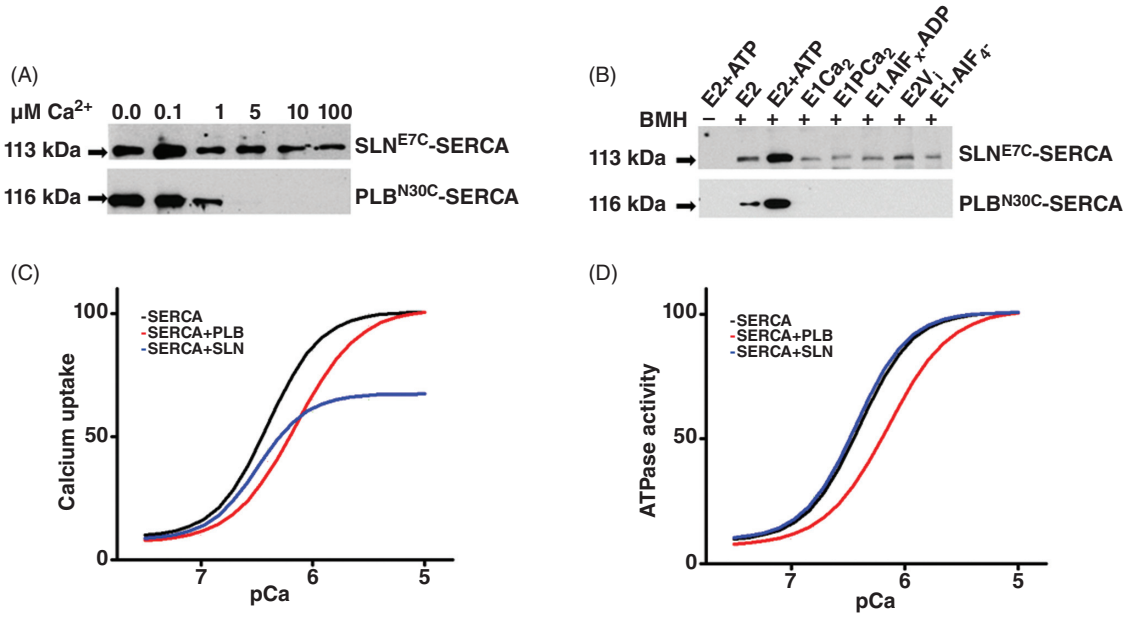

Figure 3 Distinct interaction and regulation of SERCA by SLN and PLB. SLN and PLB binding to SERCA as detected by protein cross-linking in the presence of different concentration of Ca^{2+}. (A) SLN is able to bind to SERCA even at high Ca^{2+} whereas PLB binding to SERCA is competed out by increasing Ca^{2+}, indicating that binding of PLB and Ca^{2+} are mutually exclusive. (B) SLN remains bound to SERCA pump during Ca^{2+} transport (binds to different SERCA kinetic states, that is, E2, E1, E1pCa^2, and E2P) and PLB only binds to Ca^{2+} free SERCA pump (103). (C) Ca^{2+} uptake assay profile shows that SLN decreases the V_{max} of SERCA Ca^{2+} transport by uncoupling. (D) SLN does not inhibit SERCA ATPase activity. PLB inhibits SERCA ATPase activity but has no effect on V_{max} of Ca^{2+} uptake. Adapted from Sahoo et al. JBC (103).

high Ca^{2+} (9, 102, 103). SLN binding does not affect SERCA ATPase activity, but decreases the net (V_{max}) Ca^{2+} uptake (Fig. 3). A notable difference is that SLN remains bound to SERCA during the entire kinetic cycle in the TM-groove. The presence of SLN in the TM groove interferes with Ca^{2+} transport into the lumen of SR, instead causing the slippage of Ca^{2+} back to the cytosol. PLB binding inhibits SERCA pump activity, whereas the binding of SLN to SERCA causes uncoupling of the SERCA pump from Ca^{2+} transport. This is a novel finding showing that SLN can promote futile cycling of SERCA at the expense of increased ATP hydrolysis and increased heat production in muscle. These studies led to the proposal that uncoupling of SERCA activity by SLN may play an important role in muscle thermogenesis (8, 9, 99).

SLN is Important for Muscle Thermogenesis

The physiological relevance of SLN was unknown and remained quite speculative for some time. It was thought that SLN was similar to PLB in function. To establish if SLN was important for Ca^{2+} homeostasis and muscle function, we developed a $SLN^{-/-}$ mouse model (6). Ablation of SLN did not affect survival or muscle growth and the mice were indistinguishable from WT controls (6). In addition, loss of SLN did not significantly modify Ca^{2+} cycling, nor did it have any detrimental effect on muscle function (6, 124). With this model, we investigated if the mice would adapt to cold exposure given that SLN was proposed to play a role in heat production through uncoupling of SERCA activity. The $SLN^{-/-}$ mice, when housed at 22°C, were able to reproduce well and maintain their body temperature ~37°C (9). It is important to highlight that in mice, BAT, via UCP1-based heat production, is an important contributor to thermogenesis (23). Adult mice possess a major BAT depot (~70% of total BAT) located between the two scapulae, called the interscapular BAT (iBAT). To study the importance of muscle-based thermogenesis, it was necessary to surgically remove the iBAT. When challenged at 4°C, the iBAT-ablated WT mice were cold sensitive but were still able to maintain body Tc (Fig. 4). In contrast, the iBAT-ablated $SLN^{-/-}$ mice were unable to maintain body temperature and subsequently developed hypothermia. A body Tc of ~25°C was reached within 6 hours of the cold challenge and the mice had to be removed to avoid cold-induced death (9). These studies suggest that SLN is an important player in muscle-based thermogenesis and whole body Tc regulation.

Although BAT is a dominant thermogenic mechanism in rodents, several studies have suggested other mechanisms of heat production. Rodents are unique in that they have high BAT content and rely primarily on BAT for their NST and temperature homeostasis. However, most large mammals, including humans, have little BAT tissue in adult life. Therefore, the relevance of BAT in large mammals is under debate. In addition, the predominant use of rodents as experimental animal

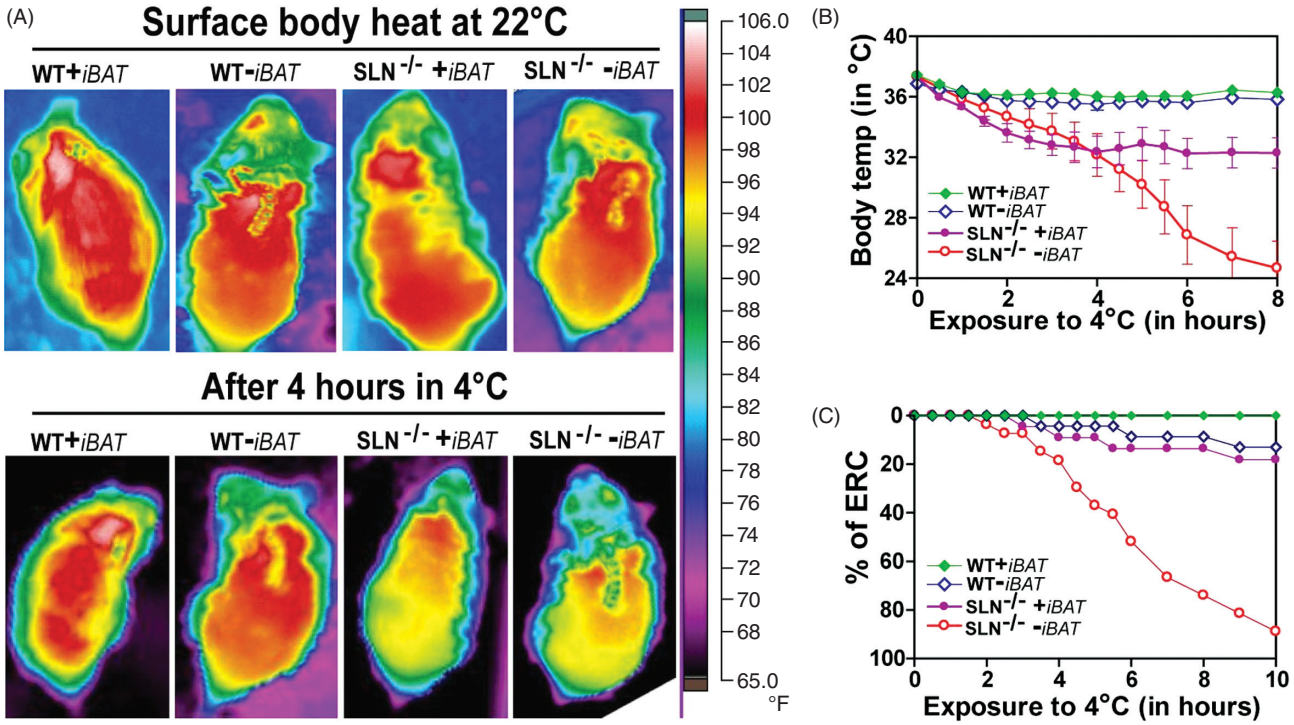

Figure 4 SLN plays a role in muscle thermogenesis. (A) Infrared imaging of surface body heat in WT and *Sln*−/− mice with or without iBAT at 22°C and 4°C. (B) Core body temperature after acute cold exposure in WT and *Sln*−/− mice, with and without iBAT. (C) Percentage of mice reaching ERC (early removal criteria) (10). The mice were removed from cold when body Tc reached 25°C. All data are means ± S.E.M. Adapted from Bal et al. Nat Medicine (9).

models has often neglected the importance of alternate thermogenic mechanisms. However, there is increasing evidence for the existence of BAT-independent sites of thermogenesis in mammals. This has become especially obvious from studies on large mammals that have limited BAT, as well as from UCP1-knockout (UCP1-KO) mice. It has been shown that UCP1-KO mice are extremely sensitive to acute cold exposure, but they can be gradually adapted to 4°C (13, 14) which argued that there must be alternate mechanisms to support thermogenesis when BAT is nonfunctional.

In a recent study, Bal et al. went on to further investigate if skeletal muscle-based NST could become hyper recruited when BAT activity is compromised by iBAT ablation in adult mice (8). This study showed that the iBAT-ablated mice were able to maintain body temperature even when they were shifted directly from 22°C to 4°C. The iBAT ablated mice-induced substantial remodeling of SR proteins, including the upregulation of SLN, SERCA2a and increased phosphorylation of RyR1, as evidence for the increased recruitment of SR Ca^{2+} cycling as the basis for NST in muscle (8). In comparison, the sham-operated animals showed a lesser remodeling of SR proteins during cold challenge. In addition, there was an increase in mitochondrial ETC proteins and oxidative metabolism in their skeletal muscles, as evidence for recruitment of NST in the skeletal muscle. Similarly, the UCP1-KO mice could be gradually cold-adapted to 4°C without any adverse effects; however, these mice showed an increase in SLN expression and upregulation of muscle-based NST (99, 101). Collectively, these studies suggested that skeletal muscle can substitute for the loss of BAT function and is energetically more costly as observed in iBAT-ablated mice that exhausted their fat depots compared to sham controls. Interestingly, most large mammals, including humans, have little functional BAT but express high levels of SLN, which suggest that these large mammals must depend on muscle-based NST when their BAT activity is diminished during development.

SLN is an Important Regulator of Energy Metabolism and is Recruited in Diet-induced Thermogenesis

Skeletal muscle is a major consumer of metabolites, including glucose and fatty acids, and is an important regulator of whole body metabolism and energy homeostasis (134, 135). Muscle heat production involving both shivering and NST must involve significant energy demand. The importance of SLN on muscle metabolism has been investigated by challenging both $SLN^{-/-}$ and SLN^{OE} mice (overexpressing SLN in all skeletal muscle tissues) by high fat diet feeding for a period of 12 weeks (9, 19, 76, 77). Interestingly, $SLN^{-/-}$ mice became significantly more obese when compared to WT controls; the obesity phenotype in $SLN^{-/-}$ was associated with poor glucose tolerance, an early sign of Type 2 diabetes (Fig. 5).

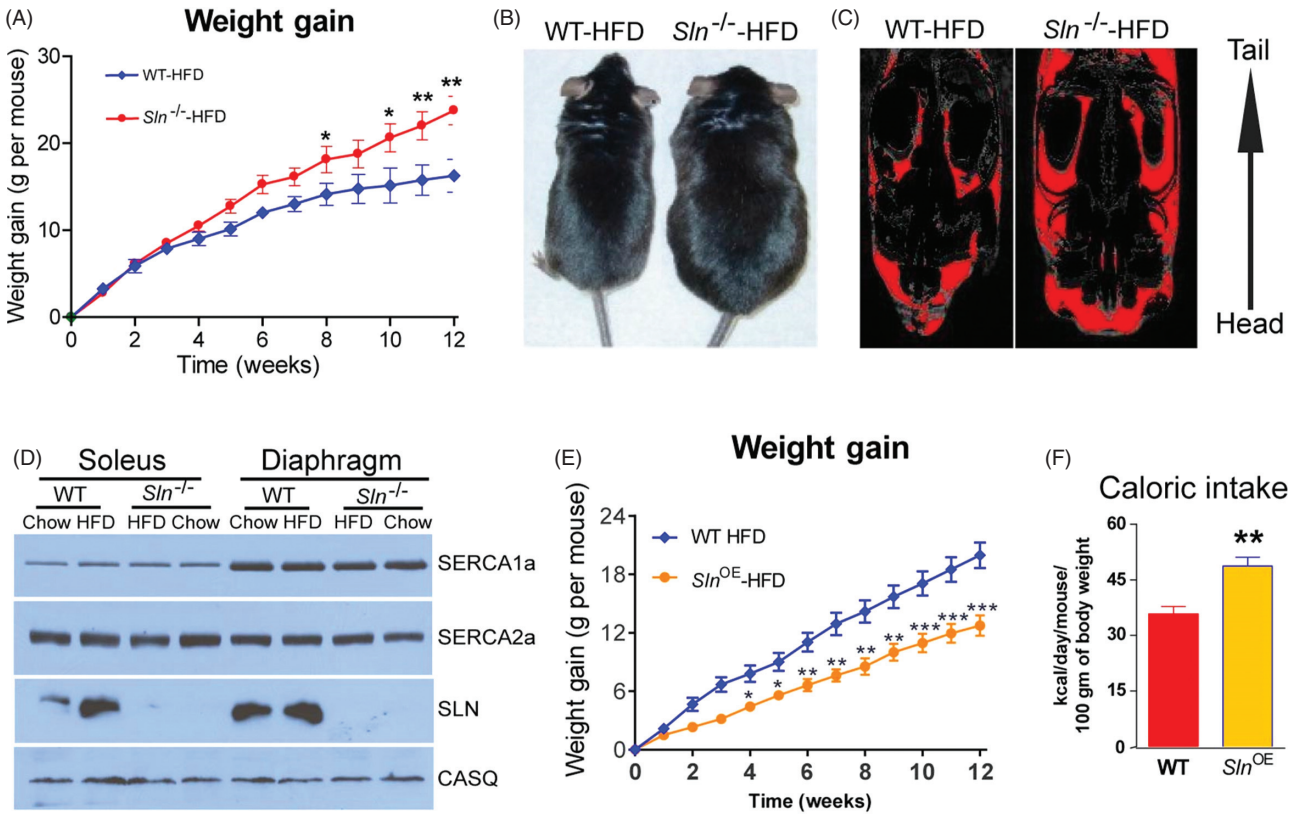

Figure 5 SLN plays an important role in whole body metabolism and obesity. Mice were fed on HFD for 12 weeks. (A and B) SLN-/- mice show significant increase in body weight. (C) MRI images showing body fat distribution in WT and *Sln*–/– after high fat diet feeding. (D) SLN protein level is increased in HFD-fed WT–Soleus. $n = 4$. (E) Overexpression of SLN provides resistance against diet induced obesity. (F) Sln^{OE} mice consumed more calories than WT during HFD feeding. All data are means ± S.E.M. Adapted from Bal et al. Nat Medicine and Maurya et al. JBC (9,76).

In contrast, WT mice were found to be less obese with upregulated SLN expression levels in skeletal muscles. It is surprising that the diet-induced obesity was not protected by the presence of BAT in $SLN^{-/-}$ mice. These studies suggest that loss of SLN in muscle is sufficient to cause decreased energy expenditure which overtime may lead to greater fat deposition and obesity (9, 19).

To further confirm that SLN was indeed responsible for the altered metabolism, Maurya et al. addressed if increasing SLN expression would lead to an increased metabolic rate using a mouse model that overexpresses SLN (under the control of the skeletal muscle α-actin promoter) in all skeletal muscle tissues (76). It must be noted that increased SLN levels had no adverse effect on muscle contractility and performance. The SLN^{OE} mice mimic a larger mammal in SLN expression pattern and express ~10-fold higher levels of SLN in skeletal muscles. These mice were also found to have a higher food intake when fed *ad libitum*. Interestingly, when SLN^{OE} mice were pair-fed along with $SLN^{-/-}$ mice, the SLN^{OE} mice lost body weight and most of their white fat content compared to WT and $SLN^{-/-}$ mice. These studies suggested that the level of SLN expression could affect energy expenditure and basal metabolic rate. It remains to be seen, however, if this is also true for larger mammals. Interestingly, when the SLN^{OE} mice were fed on HFD for 12 weeks, they were more resistant to weight gain and were significantly less obese than their WT counterparts, despite consuming more calories. Furthermore, it was noted that the energy expenditure of SLN^{OE} mice was significantly higher during the night (active phase of mice). In addition, higher oxygen consumption was also observed in isolated muscle samples from these mice. An important finding is that the SLN^{OE} mice did not exhibit any metabolic abnormalities associated with diet-induced obesity, including glucose intolerance and hyperlipidemia. Remarkably, the SLN^{OE} mice fed on HFD showed a striking increase in mitochondrial content, especially in fast glycolytic skeletal muscles like the tibialis anterior (TA) and extensor digitorum longus (EDL). Mitochondria in SLN^{OE} muscle were also larger with intricate and densely packed cristae. Further, the fast-twitch muscle TA from SLN^{OE} mice showed a higher oxidative capacity compared to that of SLN-KO muscle. In addition, SLN overexpression increased the expression levels of key transcriptional regulators (PPARδ, PGC1α and NRFs) involved in mitochondrial biogenesis and several genes involved in fatty acid oxidation. These novel findings suggest that SLN is an important regulator of muscle metabolism and plays a role in diet overload-induced adaptive thermogenesis. Further work is necessary, however, to

document how SLN is recruited during diet-induced thermogenesis and if this involves diet-induced alterations in neurohormonal signaling mechanisms.

The Role of SR-Mitochondrial Cross Talk in Energy Metabolism

In skeletal muscle, mitochondria exist within the I-band, adjacent to Ca^{2+} release units at the A-I band junctions and within the intermyofibrillar space (43, 98). Some studies have suggested that there is physical coupling between the SR and mitochondria (2, 29, 42, 110). While this is a possibility, mitochondria can also sense and take up cytosolic Ca^{2+} through the mitochondrial uniporter (MCU) and Voltage-dependent anion channel (VDAC) (47, 74, 89, 94, 128). Several recent studies have shown that mitochondrial Ca^{2+} oscillates with cytosolic Ca^{2+} release and removal (27, 68, 86). Ca^{2+} release from the SR is primarily responsible for the dynamic changes in cytosolic Ca^{2+} during rest and exercise (36, 55, 95, 107). Changes in cytosolic Ca^{2+} serve not only as a signal for muscle contraction but also for mitochondrial metabolism (18, 37, 46, 53, 54, 58, 71, 78, 113). It is known that changes in cytosolic Ca^{2+} during rest versus exercise can be decoded by each individual mitochondrion to adjust to the local metabolic demand. The presence of SLN can further enhance this SR-mitochondrial cross-talk by altering cytosolic Ca^{2+} dynamics and increasing Ca^{2+} entry into the mitochondria. Interestingly, studies conducted on the SLN^{OE} mouse model suggest that SLN overexpression is beneficial to muscle physiology; that is, higher levels of SLN promote whole animal endurance capacity and energetics (111). These studies show that SLN overexpression enhances fatigue resistance in fast and slow isolated muscles without compromising force during isometric conditions. This is a novel finding which suggest that increasing SLN expression/activity has primed the muscle

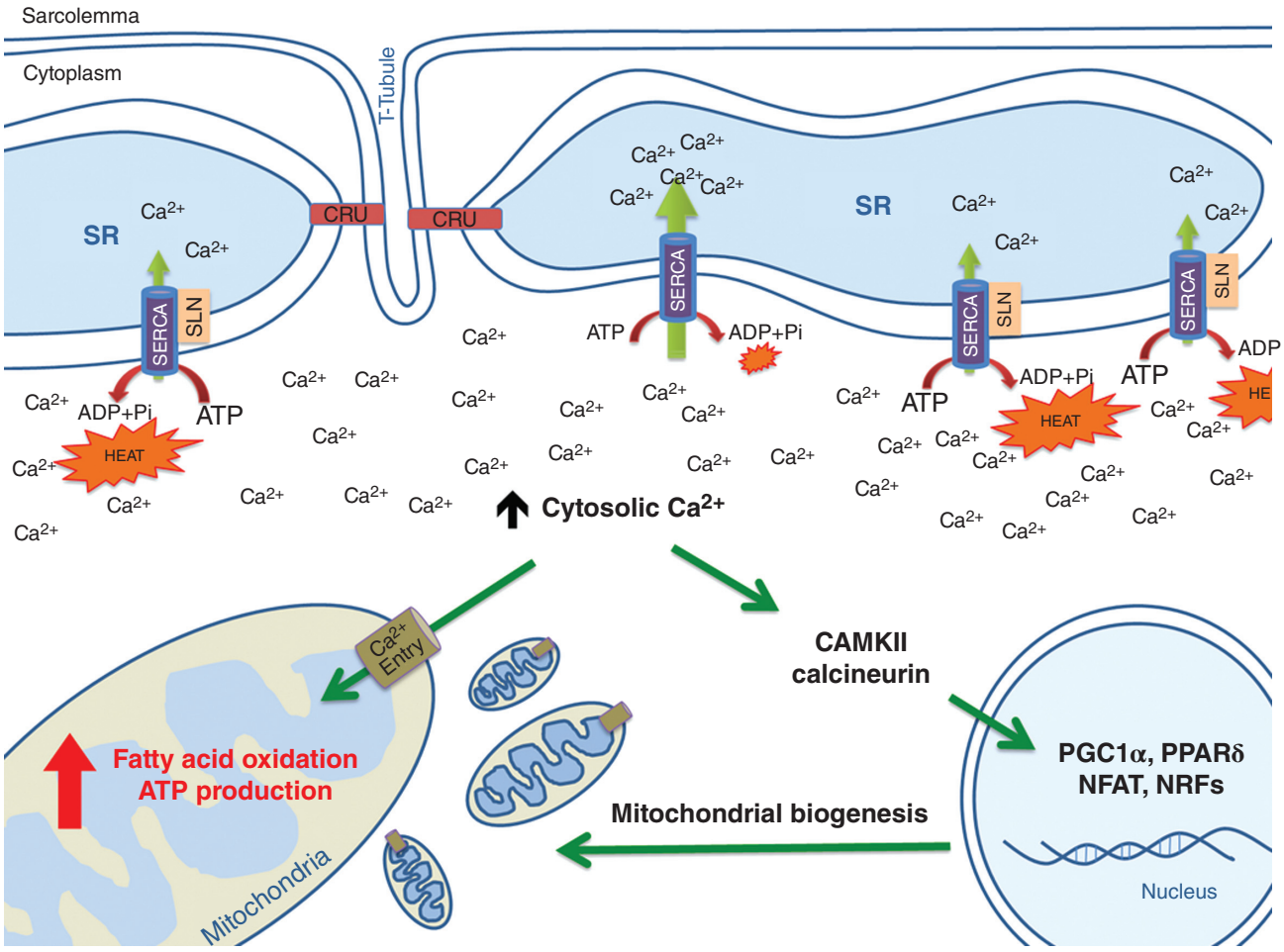

Figure 6 Proposed model showing how SLN/SERCA interaction leads to increased mitochondrial biogenesis and oxidative metabolism. SERCA uses ATP hydrolysis to actively transport Ca^{2+} from the cytosol into the sarcoplasmic reticulum lumen. SLN binding to SERCA causes uncoupling of Ca^{2+} transport from ATP hydrolysis. Uncoupling of SERCA leads to futile cycling of the pump and increased ATP hydrolysis/heat production, thereby creating energy demand. Decreased SERCA mediated Ca^{2+} uptake increases the cytosolic Ca^{2+}, which promotes Ca^{2+} entry into mitochondria, activating mitochondrial oxidative metabolism and ATP synthesis. An increase in cytosolic Ca^{2+} leads to activation of Ca^{2+}-dependent signaling pathways and nuclear transcription factors PGC1α, and PPARδ promoting mitochondrial biogenesis. CRU, calcium release unit.

to respond to increased metabolic demand without causing a switch in muscle fiber type (111). These studies suggest that during prolonged muscle activity, such as in exercise, an increase in cytosolic Ca^{2+} can serve as a metabolic signal and increase oxidative capacity through activation of metabolic enzymes that are Ca^{2+} sensitive, and this can boost ATP synthesis during high demand. It has been consistently shown that changes in Ca^{2+} dynamics in specific cytosolic compartments can activate powerful Ca^{2+}-dependent signaling pathways, involving kinases and phosphatases. It is well established that activation of CAMKII and calcineurin can result in spiraling cascades of signaling resulting in the activation of a variety of nuclear and mitochondrial transcription regulators to increase mitochondrial biogenesis during high energy demand states (31, 32, 51, 52, 69, 79, 130). Thus, we propose that SLN acts as an enhancer of oxidative metabolism and can serve as an attractive target to increase energy metabolism in sedentary individuals (Fig. 6).

Conclusion

Skeletal muscle is the largest organ in the body and can consume significant amounts of energy on a daily basis. It is a key determinant of basal metabolic rate and has the ability to exhaust the body's energy sources, including fat depots, during intense activity. Despite this ability, obesity is increasing at an alarming rate across the globe and is considered to be the major contributor to the increase in diabetes, coronary heart disease, cancer, and neurological diseases that are burdening the health care system. Obesity results from an energy imbalance; largely due to increased consumption of a caloric-rich diet and limited physical activity due to change in life style. There are no effective treatments to reduce obesity other than caloric restriction and exercise which are difficult to enforce on a daily basis. It is imperative that we identify mechanisms and targets to increase energy expenditure pharmacologically as this will become critical under conditions where physical activity may be limited. The discovery of SLN as an uncoupler of the SERCA pump is a promising target to increase energy expenditure in muscle. Our studies showing that increasing SLN expression/activity can be beneficial to muscle metabolism are a significant finding and encourage us to continue to explore this as a possible target for therapeutics. However, there are still many unanswered questions when it comes to SLN: (i) its exact mechanism of interaction and how it uncouples SERCA activity, (ii) if SLN uncoupling of SERCA is sufficient to cause increased energy demand and activation of mitochondrial metabolism or is there a need for an external signal such as cold, (iii) and most importantly, if this mechanism can be effectively targeted using small molecules to increase energy expenditure in humans. Despite these outstanding questions, skeletal muscle represents the most attractive target due to its enormous capacity to affect metabolic rate quickly and efficiently. Even adjunct therapy with exercise could be an effective way to increase energy expenditure and regulate whole body energy metabolism. Future research should aim at discovering mechanisms that increase basal metabolism as well as discovering small compounds that could increase muscle metabolism several folds.

Acknowledgements

This work was supported in part by NIDDK-National Institutes of Health Grants R01 DK098240–01, American Diabetes Association Basic Science Research Award (7-13-BS-131) (to M. Periasamy.) KO1 Award DK102772 (Naresh C. Bal), AHA Postdoctoral fellowship 14POST200380847 (Sanjaya K. Sahoo). We thank Anthony for proofreading the article. The authors declare that they have no conflicts of interest with the contents of this article.

References

1. Afara MR, Trieber CA, Glaves JP, Young HS. Rational design of peptide inhibitors of the sarcoplasmic reticulum calcium pump. *Biochemistry* 45: 8617-8627, 2006.
2. Ainbinder A, Boncompagni S, Protasi F, Dirksen RT. Role of Mitofusin-2 in mitochondrial localization and calcium uptake in skeletal muscle. *Cell Calcium* 57: 14-24, 2015.
3. Anderson DM, Anderson KM, Chang C-L, Makarewich CA, Nelson BR, McAnally JR, Kasaragod P, Shelton JM, Liou J, Bassel-Duby R. A micropeptide encoded by a putative long noncoding RNA regulates muscle performance. *Cell* 160: 595-606, 2015.
4. Arruda AP, Nigro M, Oliveira GM, de Meis L. Thermogenic activity of Ca^{2+}-ATPase from skeletal muscle heavy sarcoplasmic reticulum: the role of ryanodine Ca^{2+} channel. *Biochim Biophys Acta* 1768: 1498-1505, 2007.
5. Babu GJ, Bhupathy P, Carnes CA, Billman GE, Periasamy M. Differential expression of sarcolipin protein during muscle development and cardiac pathophysiology. *J Mol Cell Cardiol* 43: 215-222, 2007.
6. Babu GJ, Bhupathy P, Timofeyev V, Petrashevskaya NN, Reiser PJ, Chiamvimonvat N, Periasamy M. Ablation of sarcolipin enhances sarcoplasmic reticulum calcium transport and atrial contractility. *Proc Natl Acad Sci U S A* 104: 17867-17872, 2007.
7. Bachman ES, Dhillon H, Zhang CY, Cinti S, Bianco AC, Kobilka BK, Lowell BB. betaAR signaling required for diet-induced thermogenesis and obesity resistance. *Science* 297: 843-845, 2002.
8. Bal NC, Maurya SK, Singh S, Wehrens XH, Periasamy M. Increased reliance on muscle-based thermogenesis upon acute minimization of brown adipose tissue function. *J Biol Chem* 291: 17247-17257, 2016.
9. Bal NC, Maurya SK, Sopariwala DH, Sahoo SK, Gupta SC, Shaikh SA, Pant M, Rowland LA, Bombardier E, Goonasekera SA. Sarcolipin is a newly identified regulator of muscle-based thermogenesis in mammals. *Nat Med* 18: 1575-1579, 2012.
10. Benziane B, Bjornholm M, Pirkmajer S, Austin RL, Kotova O, Viollet B, Zierath JR, Chibalin AV. Activation of AMP-activated protein kinase stimulates Na+,K+-ATPase activity in skeletal muscle cells. *J Biol Chem* 287: 23451-23463, 2012.
11. Berg F, Gustafson U, Andersson L. The uncoupling protein 1 gene (UCP1) is disrupted in the pig lineage: A genetic explanation for poor thermoregulation in piglets. *PLoS Genet* 2: e129, 2006.
12. Bers DM. Cardiac excitation–contraction coupling. *Nature* 415: 198-205, 2002.
13. Bers DM, Stiffel VM. Ratio of ryanodine to dihydropyridine receptors in cardiac and skeletal muscle and implications for EC coupling. *Am J Physiol Cell Physiol* 264: C1587-C1593, 1993.
14. Bhupathy P, Babu GJ, Periasamy M. Sarcolipin and phospholamban as regulators of cardiac sarcoplasmic reticulum Ca^{2+} ATPase. *J Mol Cell Cardiol* 42: 903-911, 2007.
15. Block BA. Thermogenesis in muscle. *Annu Rev Physiol* 56: 535-577, 1994.
16. Block BA, Imagawa T, Campbell KP, Franzini-Armstrong C. Structural evidence for direct interaction between the molecular components of the transverse tubule/sarcoplasmic reticulum junction in skeletal muscle. *J Cell Biol* 107: 2587-2600, 1988.

17. Block BA, O'Brien J, Meissner G. Characterization of the sarcoplasmic reticulum proteins in the thermogenic muscles of fish. *J Cell Biol* 127: 1275-1287, 1994.
18. Boerries M, Most P, Gledhill JR, Walker JE, Katus HA, Koch WJ, Aebi U, Schoenenberger CA. Ca^{2+} -dependent interaction of S100A1 with F1-ATPase leads to an increased ATP content in cardiomyocytes. *Mol Cell Biol* 27: 4365-4373, 2007.
19. Bombardier E, Smith IC, Gamu D, Fajardo VA, Vigna C, Sayer RA, Gupta SC, Bal NC, Periasamy M, Tupling AR. Sarcolipin trumps beta-adrenergic receptor signaling as the favored mechanism for muscle-based diet-induced thermogenesis. *FASEB J* 27: 3871-3878, 2013.
20. Bouchama A, Hussain S. Malignant hyperthermia. *Lancet* 352: 1785, 1998.
21. Bublitz M, Morth JP, Nissen P. P-type ATPases at a glance. *J Cell Sci* 124: 2515-2519, 2011.
22. Cambon B, Reyne Y, Nougues J. In vitro induction of UCP1 mRNA in preadipocytes from rabbit considered as a model of large mammals brown adipose tissue development: importance of PPARgamma agonists for cells isolated in the postnatal period. *Mol Cell Endocrinol* 146: 49-58, 1998.
23. Cannon B, Nedergaard J. Brown adipose tissue: Function and physiological significance. *Physiol Rev* 84: 277-359, 2004.
24. Cannon B, Nedergaard J. Metabolic consequences of the presence or absence of the thermogenic capacity of brown adipose tissue in mice (and probably in humans). *Int J Obes* 34(Suppl 1): S7-S16, 2010.
25. Casteilla L, Champigny O, Bouillaud F, Robelin J, Ricquier D. Sequential changes in the expression of mitochondrial protein mRNA during the development of brown adipose tissue in bovine and ovine species. Sudden occurrence of uncoupling protein mRNA during embryogenesis and its disappearance after birth. *Biochem J* 257: 665-671, 1989.
26. Castellani JW, Tipton MJ. Cold stress effects on exposure tolerance and exercise performance. *Compr Physiol* 6: 443-469, 2015.
27. Chacon E, Ohata H, Harper IS, Trollinger DR, Herman B, Lemasters JJ. Mitochondrial free calcium transients during excitation-contraction coupling in rabbit cardiac myocytes. *FEBS Lett* 382: 31-36, 1996.
28. Chaffee RR, Roberts JC. Temperature acclimation in birds and mammals. *Annu Rev Physiol* 33: 155-202, 1971.
29. Chen Y, Csordas G, Jowdy C, Schneider TG, Csordas N, Wang W, Liu Y, Kohlhaas M, Meiser M, Bergem S, Nerbonne JM, Dorn GW, II, Maack C. Mitofusin 2-containing mitochondrial-reticular microdomains direct rapid cardiomyocyte bioenergetic responses via interorganelle $Ca(2^+)$ crosstalk. *Circ Res* 111: 863-875, 2012.
30. Cheshire WP, Jr. Thermoregulatory disorders and illness related to heat and cold stress. *Auton Neurosci* 196: 91-104, 2016.
31. Chin ER. Role of Ca^{2+}/calmodulin-dependent kinases in skeletal muscle plasticity. *J Appl Physiol* 99: 414-423, 2005.
32. Combes A, Dekerle J, Webborn N, Watt P, Bougault V, Daussin FN. Exercise-induced metabolic fluctuations influence AMPK, p38-MAPK and CaMKII phosphorylation in human skeletal muscle. *Physiol Rep* 3(9): pii: e12462, 2015.
33. de Meis L, Arruda AP, Carvalho DP. Role of sarco/endoplasmic reticulum $Ca(2^+)$-ATPase in thermogenesis. *Biosci Rep* 25: 181-190, 2005.
34. de Meis L, Arruda AP, da-Silva WS, Reis M, Carvalho DP. The thermogenic function of the sarcoplasmic reticulum Ca^{2+}-ATPase of normal and hyperthyroid rabbit. *Ann N Y Acad Sci* 986: 481-488, 2003.
35. de Meis L, Oliveira GM, Arruda AP, Santos R, Costa RM, Benchimol M. The thermogenic activity of rat brown adipose tissue and rabbit white muscle Ca^{2+}-ATPase. *IUBMB Life* 57: 337-345, 2005.
36. Dedkova EN, Blatter LA. Mitochondrial Ca^{2+} and the heart. *Cell Calcium* 44: 77-91, 2008.
37. Denton RM, Randle PJ, Martin BR. Stimulation by calcium ions of pyruvate dehydrogenase phosphate phosphatase. *Biochem J* 128: 161-163, 1972.
38. Eldershaw TP, Duchamp C, Ye J, Clark MG, Colquhoun EQ. Potential for non-shivering thermogenesis in perfused chicken (Gallus domesticus) muscle. *Comp Biochem Physiol A* 117: 545-554, 1997.
39. Enerback S, Jacobsson A, Simpson EM, Guerra C, Yamashita H, Harper ME, Kozak LP. Mice lacking mitochondrial uncoupling protein are cold-sensitive but not obese. *Nature* 387: 90-94, 1997.
40. Fagher B, Liedholm H, Monti M, Moritz U. Thermogenesis in human skeletal muscle as measured by direct microcalorimetry and muscle contractile performance during beta-adrenoceptor blockade. *Clin Sci* 70: 435-441, 1986.
41. Ferrannini E, Simonson DC, Katz LD, Reichard G, Jr., Bevilacqua S, Barrett EJ, Olsson M, DeFronzo RA. The disposal of an oral glucose load in patients with non-insulin-dependent diabetes. *Metabolism* 37: 79-85, 1988.
42. Franzini-Armstrong C. ER-mitochondria communication. How privileged? *Physiology* 22: 261-268, 2007.
43. Franzini-Armstrong C, Boncompagni S. The evolution of the mitochondria-to-calcium release units relationship in vertebrate skeletal muscles. *J Biomed Biotechnol* 2011: 830573, 2011.
44. Franzini-Armstrong C, Jorgensen AO. Structure and development of EC coupling units in skeletal muscle. *Annu Rev Physiol* 56: 509-534, 1994.
45. Franzini-Armstrong C, Protasi F, Ramesh V. Comparative ultrastructure of Ca^{2+} release units in skeletal and cardiac muscle. *Ann N Y Acad Sci* 853: 20-30, 1998.
46. Gabriel JL, Plaut GW. Inhibition of bovine heart NAD-specific isocitrate dehydrogenase by reduced pyridine nucleotides: Modulation of inhibition by ADP, NAD^+, Ca^{2+}, citrate, and isocitrate. *Biochemistry* 23: 2773-2778, 1984.
47. Garcia-Perez C, Schneider TG, Hajnoczky G, Csordas G. Alignment of sarcoplasmic reticulum-mitochondrial junctions with mitochondrial contact points. *Am J Physiol Heart Circ Physiol* 301: H1907-H1915, 2011.
48. Guerra C, Koza RA, Walsh K, Kurtz DM, Wood PA, Kozak LP. Abnormal nonshivering thermogenesis in mice with inherited defects of fatty acid oxidation. *J Clin Invest* 102: 1724-1731, 1998.
49. Haman F. Shivering in the cold: from mechanisms of fuel selection to survival. *J Appl Physiol* 100: 1702-1708, 2006.
50. He ZH, Bottinelli R, Pellegrino MA, Ferenczi MA, Reggiani C. ATP consumption and efficiency of human single muscle fibers with different myosin isoform composition. *Biophys J* 79: 945-961, 2000.
51. Herbst EA, Roussakis C, Matravadia S, Holloway GP. Chronic treadmill running does not enhance mitochondrial oxidative capacity in the cortex or striatum. *Metabolism* 64: 1419-1425, 2015.
52. Holloszy JO. Regulation of mitochondrial biogenesis and GLUT4 expression by exercise. *Compr Physiol* 1: 921-940, 2011.
53. Hopper RK, Carroll S, Aponte AM, Johnson DT, French S, Shen RF, Witzmann FA, Harris RA, Balaban RS. Mitochondrial matrix phosphoproteome: effect of extra mitochondrial calcium. *Biochemistry* 45: 2524-2536, 2006.
54. Hubbard MJ, McHugh NJ. Mitochondrial ATP synthase F1-beta-subunit is a calcium-binding protein. *FEBS Lett* 391: 323-329, 1996.
55. Huser J, Blatter LA, Sheu SS. Mitochondrial calcium in heart cells: beat-to-beat oscillations or slow integration of cytosolic transients? *J Bioenerg Biomembr* 32: 27-33, 2000.
56. Iaizzo PA, Kehler CH, Zink RS, Belani KG, Sessler DI. Thermal response in acute porcine malignant hyperthermia. *Anesth Analg* 82: 782-789, 1996.
57. Janssen I, Heymsfield SB, Wang ZM, Ross R. Skeletal muscle mass and distribution in 468 men and women aged 18-88 yr. *J Appl Physiol* 89: 81-88, 2000.
58. Jouaville LS, Pinton P, Bastianutto C, Rutter GA, Rizzuto R. Regulation of mitochondrial ATP synthesis by calcium: Evidence for a long-term metabolic priming. *Proc Natl Acad Sci U S A* 96: 13807-13812, 1999.
59. Joubert R, Metayer-Coustard S, Crochet S, Cailleau-Audouin E, Dupont J, Duclos MJ, Tesseraud S, Collin A. Regulation of the expression of the avian uncoupling protein 3 by isoproterenol and fatty acids in chick myoblasts: Possible involvement of AMPK and PPARalpha? *Am J Physiol Regul Integr Comp Physiol* 301: R201-R208, 2011.
60. Karan SM, Lojeski EW. Skeletal muscle is responsible for heat production in porcine malignant hyperthermia. *Anesth Analg* 83: 1133-1134, 1996.
61. Kelley DE. Skeletal muscle fat oxidation: Timing and flexibility are everything. *J Clin Invest* 115: 1699-1702, 2005.
62. Kjelstrup S, de Meis L, Bedeaux D, Simon JM. Is the Ca^{2+}-ATPase from sarcoplasmic reticulum also a heat pump? *Eur Biophys J* 38: 59-67, 2008.
63. Koss KL, Kranias EG. Phospholamban: A prominent regulator of myocardial contractility. *Circ Res* 79: 1059-1063, 1996.
64. Lean ME, James WP, Jennings G, Trayhurn P. Brown adipose tissue uncoupling protein content in human infants, children and adults. *Clin Sci* 71: 291-297, 1986.
65. Lefaucheur L, Ecolan P, Lossec G, Gabillard JC, Butler-Browne GS, Herpin P. Influence of early postnatal cold exposure on myofiber maturation in pig skeletal muscle. *J Muscle Res Cell Motil* 22: 439-452, 2001.
66. Lomax MA, Sadiq F, Karamanlidis G, Karamitri A, Trayhurn P, Hazlerigg DG. Ontogenic loss of brown adipose tissue sensitivity to beta-adrenergic stimulation in the ovine. *Endocrinology* 148: 461-468, 2007.
67. Londraville RL, Cramer TD, Franck JP, Tullis A, Block BA. Cloning of a neonatal calcium atpase isoform (SERCA 1B) from extraocular muscle of adult blue marlin (Makaira nigricans). *Comp Biochem Physiol Biochem Mol Biol* 127: 223-233, 2000.
68. Lu X, Ginsburg KS, Kettlewell S, Bossuyt J, Smith GL, Bers DM. Measuring local gradients of intramitochondrial $[Ca(2^+)]$ in cardiac myocytes during sarcoplasmic reticulum $Ca(2^+)$ release. *Circ Res* 112: 424-431, 2013.
69. Luo M, Joiner ML. Stress response signaling pathways may lead to mitochondrial biogenesis. *Diabetes* 63: 1831-1832, 2014.
70. Luo W, Grupp IL, Harrer J, Ponniah S, Grupp G, Duffy JJ, Doetschman T, Kranias EG. Targeted ablation of the phospholamban gene is

associated with markedly enhanced myocardial contractility and loss of beta-agonist stimulation. *Circ Res* 75: 401-409, 1994.
71. MacDonald MJ, Brown LJ. Calcium activation of mitochondrial glycerol phosphate dehydrogenase restudied. *Arch Biochem Biophys* 326: 79-84, 1996.
72. MacLennan DH, Kranias EG. Phospholamban: A crucial regulator of cardiac contractility. *Nat Rev Mol Cell Biol* 4: 566-577, 2003.
73. Mall S, Broadbridge R, Harrison SL, Gore MG, Lee AG, East JM. The presence of sarcolipin results in increased heat production by Ca^{2+}-ATPase. *J Biol Chem* 281: 36597-36602, 2006.
74. Mammucari C, Raffaello A, Vecellio Reane D, Rizzuto R. Molecular structure and pathophysiological roles of the Mitochondrial Calcium Uniporter. *Biochim Biophys Acta* 1863: 2457-2464, 2016.
75. Matthias A, Ohlson KB, Fredriksson JM, Jacobsson A, Nedergaard J, Cannon B. Thermogenic responses in brown fat cells are fully UCP1-dependent. UCP2 or UCP3 do not substitute for UCP1 in adrenergically or fatty scid-induced thermogenesis. *J Biol Chem* 275: 25073-25081, 2000.
76. Maurya SK, Bal NC, Sopariwala DH, Pant M, Rowland LA, Shaikh SA, Periasamy M. Sarcolipin is a key determinant of the basal metabolic rate, and its overexpression enhances energy expenditure and resistance against diet-induced obesity. *J Biol Chem* 290: 10840-10849, 2015.
77. Maurya SK, Periasamy M. Sarcolipin is a novel regulator of muscle metabolism and obesity. *Pharmacol Res* 102: 270-275, 2015.
78. McCormack JG, Denton RM. The role of mitochondrial Ca^{2+} transport and matrix Ca^{2+} in signal transduction in mammalian tissues. *Biochim Biophys Acta* 1018: 287-291, 1990.
79. Michel RN, Chin ER, Chakkalakal JV, Eibl JK, Jasmin BJ. Ca^{2+}/calmodulin-based signalling in the regulation of the muscle fibre phenotype and its therapeutic potential via modulation of utrophin A and myostatin expression. *Appl Physiol Nutr Metab* 32: 921-929, 2007.
80. Møller JV, Olesen C, Winther A-ML, Nissen P. The sarcoplasmic Ca^{2+}-ATPase: Design of a perfect chemi-osmotic pump. *Q Rev Biophys* 43: 501-566, 2010.
81. Morrissette JM, Franck JP, Block BA. Characterization of ryanodine receptor and Ca^{2+}-ATPase isoforms in the thermogenic heater organ of blue marlin (Makaira nigricans). *J Exp Biol* 206: 805-812, 2003.
82. Murgia M, Nagaraj N, Deshmukh AS, Zeiler M, Cancellara P, Moretti I, Reggiani C, Schiaffino S, Mann M. Single muscle fiber proteomics reveals unexpected mitochondrial specialization. *EMBO Rep* 16: 387-395, 2015.
83. Nelson BR, Makarewich CA, Anderson DM, Winders BR, Troupes CD, Wu F, Reese AL, McAnally JR, Chen X, Kavalali ET. A peptide encoded by a transcript annotated as long noncoding RNA enhances SERCA activity in muscle. *Science* 351: 271-275, 2016.
84. Newman SA. Thermogenesis, muscle hyperplasia, and the origin of birds. *Bioessays* 33: 653-656, 2011.
85. O'Brien J, Block BA. Effects of Ca^{2+} on oxidative phosphorylation in mitochondria from the thermogenic organ of marlin. *J Exp Biol* 199: 2679-2687, 1996.
86. Ohata H, Chacon E, Tesfai SA, Harper IS, Herman B, Lemasters JJ. Mitochondrial Ca^{2+} transients in cardiac myocytes during the excitation-contraction cycle: Effects of pacing and hormonal stimulation. *J Bioenerg Biomembr* 30: 207-222, 1998.
87. Olesen C, Picard M, Winther A-ML, Gyrup C, Morth JP, Oxvig C, Møller JV, Nissen P. The structural basis of calcium transport by the calcium pump. *Nature* 450: 1036-1042, 2007.
88. Olesen C, Sørensen TL-M, Nielsen RC, Møller JV, Nissen P. Dephosphorylation of the calcium pump coupled to counterion occlusion. *Science* 306: 2251-2255, 2004.
89. Pan X, Liu J, Nguyen T, Liu C, Sun J, Teng Y, Fergusson MM, Rovira, II, Allen M, Springer DA, Aponte AM, Gucek M, Balaban RS, Murphy E, Finkel T. The physiological role of mitochondrial calcium revealed by mice lacking the mitochondrial calcium uniporter. *Nat Cell Biol* 15: 1464-1472, 2013.
90. Pant M, Bal NC, Periasamy M. Cold adaptation overrides developmental regulation of sarcolipin expression in mice skeletal muscle: SOS for muscle-based thermogenesis? *J Exp Biol* 218(Pt 15): 2321-2325, 2015.
91. Periasamy M, Kalyanasundaram A. SERCA pump isoforms: Their role in calcium transport and disease. *Muscle Nerve* 35: 430-442, 2007.
92. Pirkmajer S, Chibalin AV. Na,K-ATPase regulation in skeletal muscle. *Am J Physiol Endocrinol Metab* 311: E1-E31, 2016.
93. Protasi F. Structural interaction between RYRs and DHPRs in calcium release units of cardiac and skeletal muscle cells. *Front Biosci* 7: d650-d658, 2002.
94. Rapizzi E, Pinton P, Szabadkai G, Wieckowski MR, Vandecasteele G, Baird G, Tuft RA, Fogarty KE, Rizzuto R. Recombinant expression of the voltage-dependent anion channel enhances the transfer of Ca^{2+} microdomains to mitochondria. *J Cell Biol* 159: 613-624, 2002.
95. Robert V, Gurlini P, Tosello V, Nagai T, Miyawaki A, Di Lisa F, Pozzan T. Beat-to-beat oscillations of mitochondrial [Ca^{2+}] in cardiac cells. *EMBO J* 20: 4998-5007, 2001.
96. Rosenberg H, Davis M, James D, Pollock N, Stowell K. Malignant hyperthermia. *Orphanet J Rare Dis* 2: 21, 2007.
97. Rosenberg H, Pollock N, Schiemann A, Bulger T, Stowell K. Malignant hyperthermia: A review. *Orphanet J Rare Dis* 10: 93, 2015.
98. Rossi AE, Boncompagni S, Wei L, Protasi F, Dirksen RT. Differential impact of mitochondrial positioning on mitochondrial $Ca(2^+)$ uptake and $Ca(2^+)$ spark suppression in skeletal muscle. *Am J Physiol Cell Physiol* 301: C1128-C1139, 2011.
99. Rowland LA, Bal NC, Kozak LP, Periasamy M. Uncoupling protein 1 and sarcolipin are required to maintain optimal thermogenesis, and loss of both systems compromises survival of mice under cold stress. *J Biol Chem* 290: 12282-12289, 2015.
100. Rowland LA, Bal NC, Periasamy M. The role of skeletal-muscle-based thermogenic mechanisms in vertebrate endothermy. *Biol Rev* 90: 1279-1297, 2015.
101. Rowland LA, Maurya SK, Bal NC, Kozak L, Periasamy M. Sarcolipin and uncoupling protein 1 play distinct roles in diet-induced thermogenesis and do not compensate for one another. *Obesity* 24(7): 1430-1433, 2016.
102. Sahoo SK, Shaikh SA, Sopariwala DH, Bal NC, Bruhn DS, Kopec W, Khandelia H, Periasamy M. The N terminus of sarcolipin plays an important role in uncoupling sarco-endoplasmic reticulum Ca^{2+}-ATPase (SERCA) ATP hydrolysis from Ca^{2+} transport. *J Biol Chem* 290: 14057-14067, 2015.
103. Sahoo SK, Shaikh SA, Sopariwala DH, Bal NC, Periasamy M. Sarcolipin protein interaction with sarco (endo) plasmic reticulum Ca^{2+} ATPase (SERCA) is distinct from phospholamban protein, and only sarcolipin can promote uncoupling of the SERCA pump. *J Biol Chem* 288: 6881-6889, 2013.
104. Schiaffino S, Reggiani C. Myosin isoforms in mammalian skeletal muscle. *J Appl Physiol* 77: 493-501, 1994.
105. Schiaffino S, Reggiani C. Molecular diversity of myofibrillar proteins: Gene regulation and functional significance. *Physiol Rev* 76: 371-423, 1996.
106. Schiaffino S, Reggiani C. Fiber types in mammalian skeletal muscles. *Physiol Rev* 91: 1447-1531, 2011.
107. Sedova M, Dedkova EN, Blatter LA. Integration of rapid cytosolic Ca^{2+} signals by mitochondria in cat ventricular myocytes. *Am J Physiol Cell Physiol* 291: C840-C850, 2006.
108. Shaikh SA, Sahoo SK, Periasamy M. Phospholamban and sarcolipin: Are they functionally redundant or distinct regulators of the Sarco (Endo) plasmic reticulum calcium ATPase? *J Mol Cell Cardiol* 91: 81-91, 2016.
109. Silva JE. Physiological importance and control of non-shivering facultative thermogenesis. *Front Biosci* 3: 352-371, 2011.
110. Song M, Dorn GW, 2nd. Mitoconfusion: Noncanonical functioning of dynamism factors in static mitochondria of the heart. *Cell Metab* 21: 195-205, 2015.
111. Sopariwala DH, Pant M, Shaikh SA, Goonasekera SA, Molkentin JD, Weisleder N, Ma J, Pan Z, Periasamy M. Sarcolipin overexpression improves muscle energetics and reduces fatigue. *J Appl Physiol* 118: 1050-1058, 2015.
112. Sørensen TL-M, Møller JV, Nissen P. Phosphoryl transfer and calcium ion occlusion in the calcium pump. *Science* 304: 1672-1675, 2004.
113. Tarasov AI, Griffiths EJ, Rutter GA. Regulation of ATP production by mitochondrial $Ca(2^+)$. *Cell Calcium* 52: 28-35, 2012.
114. Teulier L, Rouanet JL, Letexier D, Romestaing C, Belouze M, Rey B, Duchamp C, Roussel D. Cold-acclimation-induced non-shivering thermogenesis in birds is associated with upregulation of avian UCP but not with innate uncoupling or altered ATP efficiency. *J Exp Biol* 213: 2476-2482, 2010.
115. Thomas SA, Palmiter RD. Thermoregulatory and metabolic phenotypes of mice lacking noradrenaline and adrenaline. *Nature* 387: 94-97, 1997.
116. Toyofuku T, Kurzydlowski K, Tada M, MacLennan D. Identification of regions in the Ca (2^+)-ATPase of sarcoplasmic reticulum that affect functional association with phospholamban. *J Biol Chem* 268: 2809-2815, 1993.
117. Toyoshima C. Structural aspects of ion pumping by Ca^{2+}-ATPase of sarcoplasmic reticulum. *Arch Biochem Biophys* 476: 3-11, 2008.
118. Toyoshima C, Inesi G. Structural basis of ion pumping by Ca^{2+}-ATPase of the sarcoplasmic reticulum. *Biochemistry* 73: 269, 2004.
119. Toyoshima C, Iwasawa S, Ogawa H, Hirata A, Tsueda J, Inesi G. Crystal structures of the calcium pump and sarcolipin in the Mg^{2+}-bound E1 state. *Nature* 495: 260-264, 2013.
120. Toyoshima C, Mizutani T. Crystal structure of the calcium pump with a bound ATP analogue. *Nature* 430: 529-535, 2004.
121. Toyoshima C, Nakasako M, Nomura H, Ogawa H. Crystal structure of the calcium pump of sarcoplasmic reticulum at 2.6 Å resolution. *Nature* 405: 647-655, 2000.
122. Toyoshima C, Nomura H. Structural changes in the calcium pump accompanying the dissociation of calcium. *Nature* 418: 605-611, 2002.

123. Toyoshima C, Nomura H, Tsuda T. Lumenal gating mechanism revealed in calcium pump crystal structures with phosphate analogues. *Nature* 432: 361-368, 2004.
124. Tupling AR, Bombardier E, Gupta SC, Hussain D, Vigna C, Bloemberg D, Quadrilatero J, Trivieri MG, Babu GJ, Backx PH. Enhanced Ca^{2+} transport and muscle relaxation in skeletal muscle from sarcolipin-null mice. *Am J Physiol Cell Physiol* 301: C841-C849, 2011.
125. Turner N, Cooney GJ, Kraegen EW, Bruce CR. Fatty acid metabolism, energy expenditure and insulin resistance in muscle. *J Endocrinol* 220: T61-T79, 2014.
126. van Laack RL, Faustman C, Sebranek JG. Pork quality and the expression of stress protein Hsp 70 in swine. *J Anim Sci* 71: 2958-2964, 1993.
127. van Marken Lichtenbelt WD, Daanen HA. Cold-induced metabolism. *Curr Opin Clin Nutr Metab Care* 6: 469-475, 2003.
128. Williams GS, Boyman L, Chikando AC, Khairallah RJ, Lederer WJ. Mitochondrial calcium uptake. *Proc Natl Acad Sci U S A* 110: 10479-10486, 2013.
129. Winther A-ML, Bublitz M, Karlsen JL, Møller JV, Hansen JB, Nissen P, Buch-Pedersen MJ. The sarcolipin-bound calcium pump stabilizes calcium sites exposed to the cytoplasm. *Nature* 495: 265-269, 2013.
130. Wright DC, Geiger PC, Han DH, Jones TE, Holloszy JO. Calcium induces increases in peroxisome proliferator-activated receptor gamma coactivator-1alpha and mitochondrial biogenesis by a pathway leading to p38 mitogen-activated protein kinase activation. *J Biol Chem* 282: 18793-18799, 2007.
131. Wu J, Cohen P, Spiegelman BM. Adaptive thermogenesis in adipocytes: Is beige the new brown? *Genes Dev* 27: 234-250, 2013.
132. Zhang L, Kelley J, Schmeisser G, Kobayashi YM, Jones LR. Complex formation between junctin, triadin, calsequestrin, and the ryanodine receptor proteins of the cardiac junctional sarcoplasmic reticulum membrane. *J Biol Chem* 272: 23389-23397, 1997.
133. Zhang Y, Carter T, Eyster K, Swanson DL. Acute cold and exercise training up-regulate similar aspects of fatty acid transport and catabolism in house sparrows (Passer domesticus). *J Exp Biol* 218: 3885-3893, 2015.
134. Zurlo F, Larson K, Bogardus C, Ravussin E. Skeletal muscle metabolism is a major determinant of resting energy expenditure. *J Clin Invest* 86: 1423-1427, 1990.
135. Zurlo F, Nemeth PM, Choksi RM, Sesodia S, Ravussin E. Whole-body energy metabolism and skeletal muscle biochemical characteristics. *Metabolism* 43: 481-486, 1994.