Trends in Endocrinology & Metabolism

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Review

Sarcolipin: A Key Thermogenic and Metabolic Regulator in Skeletal Muscle

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Skeletal muscle constitutes ~40% of body mass and has the capacity to play a major role as thermogenic, metabolic, and endocrine organ. In addition to shivering, muscle also contributes to nonshivering thermogenesis via futile sarcoplasmic/endoplasmic reticulum Ca^{2+} ATPase (SERCA) activity. Sarcolipin (SLN), a regulator of SERCA activity in muscle, plays an important role in regulating muscle thermogenesis and metabolism. Uncoupling of SERCA by SLN increases ATP hydrolysis and heat production, and contributes to temperature homeostasis. SLN also affects whole-body metabolism and weight gain in mice, and is upregulated in various muscle diseases including muscular dystrophy, suggesting a role for SLN during increased metabolic demand. In this review we also highlight the physiological roles of skeletal muscle beyond contraction.

Skeletal Muscle Beyond Contraction

Fascination with skeletal muscle anatomy and function dates back to several centuries as seen from Michelangelo's classical paintings and Leonardo da Vinci's vivid sketches depicting architecture of human skeletal muscle. During the 20th century, scientists were more interested in the mechanics of muscle contraction, which led to the identification of many contractile proteins [1] and the sliding filament theory as the basis for muscle movement [2]. A large body of research was devoted to understanding striated muscle structure, organization, fiber types, and performance. There has also been much interest in muscle growth (body building), hypertrophy, and adaptation to exercise due to its relevance to health and sports medicine [3,4]. Despite significant advances in our understanding of muscle as a contractile organ, these early studies placed less emphasis on the importance of skeletal muscle in **thermogenesis** (see Glossary) and metabolism. These studies assumed that heat production and metabolic activity in the skeletal muscle are primarily due to contractile activity. However, emerging studies suggest that some of these functions are not exclusively dependent on muscle contraction. These studies provide evidence that muscle can also function as a thermogenic, metabolic, and endocrine organ (Box 1, Figure 1). Table 1 shows study models (key genetically modified mouse models and cell lines) used to understand skeletal muscle functions beyond contraction.

Skeletal muscle occupies ~40% of mammalian body mass and consumes more than 70% of metabolites during extreme physiological demands such as exercise [5,6]. Therefore, muscle can play a dominant role in the regulation of the whole-body metabolic rate, and any perturbation in muscle health can have significant metabolic consequence. Pioneering studies in endothermic fish and birds suggested that **sarcoplasmic reticulum (SR)** calcium cycling in muscle can be activated to produce heat in these organisms and an increase in muscle metabolism is required to support this mechanism [7]. Recent studies demonstrated that **sarcolipin (SLN)** a regulator

Trends

Recent studies have highlighted that skeletal muscle plays important roles as a metabolic, thermogenic, and endocrine organ.

It had been known that the sarcoplasmic reticulum calcium ion transport is recruited in muscle-based thermogenesis, but the mechanistic details were missing.

Uncoupling of sarcoplasmic/endoplasmic reticulum Ca^{2+} ATPase (SERCA) by sarcolipin (SLN) increases ATP hydrolysis, enhances muscle metabolism, and promotes heat generation. It also alters cytosolic calcium levels, which act as a powerful signal to regulate mitochondrial metabolism and architecture.

Overexpression of SLN in muscle improves muscle energetics, providing resistance to diet-induced obesity.

SLN protein levels are upregulated during diet overload and cold adaptation and in dystrophic skeletal muscle, suggesting that SLN might be involved in promoting oxidative metabolism during conditions of metabolic stress.

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Box 1. Muscle Also Serves as an Important Metabolic and Endocrine Organ

Muscle is a major consumer of metabolites due to constant maintenance and renewal of its mass, therefore serves as a key determinant of the basal metabolic rate, and metabolic homeostasis. For example, under normal physiological conditions skeletal muscle crosstalk with liver is responsible for regulating glucose levels in the body. However, during intense exercise, muscle metabolic rate can be swiftly upregulated to utilize a substantial percentage of whole body glucose [5]. Additional metabolic flexibility allows muscle to use fat or ketones depending on availability and physiological demand [76]. During infection or starvation, muscle can switch to fatty acid utilization allowing continued glucose utilization by other organs like brain that rely on it exclusively. When challenged with prolonged starvation, skeletal muscle can trigger degradation of its proteins to provide amino acids for gluconeogenesis in liver (Figure 1). The role of skeletal muscle in the whole body metabolic demand is also supported by recent studies using high-precision **indirect calorimetry** that measured oxygen consumption in resting forearm of human subjects and demonstrated that skeletal muscle accounts for at least 20% of whole body oxygen uptake [6]. These results provided evidence that energy expenditure in skeletal muscle is much higher than accounted by only the contractile status of the muscles recruited. Skeletal muscle also serves as an important endocrine organ and secretes several '**myokines**' in a muscle activity-dependent or -independent manner. For example, it is known that interleukin 6 (IL-6) level is increased several folds in the circulation during intense physical exercise [77]. IL-6 plays a key role in the substrate utilization by enhancing lipolysis in white adipose tissue (WAT), glycolysis in the liver, and insulin secretion from the pancreas. The importance of IL-1, IL-8, and IL-15 as myokines is also becoming clear during basal metabolism, diet overload, exercise, and cold adaptation [78]. More recent studies have identified several other myokines including leukemia inhibitory factor (LIF), insulin-like growth factor (IGF)-1, fibroblast growth factor (FGF)-2, and FGF-21, that play an important role in energy homeostasis by regulating function of multiple organs. The role of these myokines in general and in particular the role of FGF-21 as a key regulator of metabolism in liver, heart, WAT, BAT, and pancreas function, is an emerging area of research [79]. A better understanding of the regulation of skeletal muscle metabolism and its crosstalk with other organs will provide insight towards manipulating whole body energy expenditure (Figure 1, Table 1).

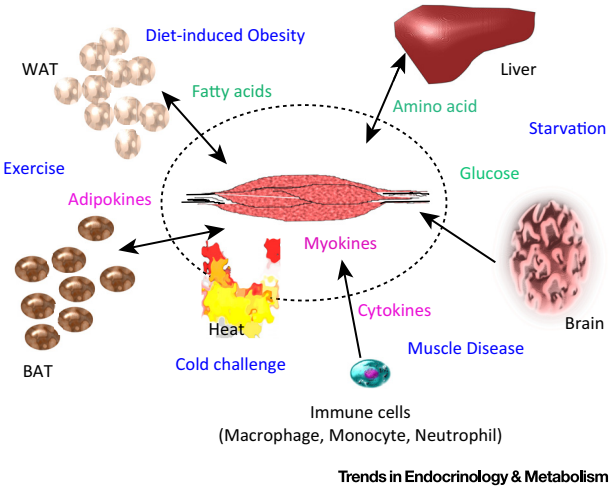

Figure 1. Skeletal Muscle Also Serves as an Important Thermogenic, Metabolic, and Endocrine Organ. Skeletal muscle is involved in both shivering and nonshivering thermogenic mechanisms, which are recruited during cold adaptation and facultative diet induced thermogenesis (fDIT). Skeletal muscle also serves as an endocrine organ by secreting several myokines to communicate with other participating organs (BAT, WAT, liver, and brain) to maintain energy homeostasis under different metabolic and pathophysiological stress conditions, including exercise, inflammation, and muscle disease. Exercise induced increase in energy expenditure has been shown to be beneficial in treating metabolic disorders. Crosstalk between adipose tissues and skeletal muscle through **adipokines** and myokines facilitate metabolic homeostasis. Neurohormonal input from the central nervous system plays a central role in coordinating muscle physiology and metabolism. Abbreviations: BAT, brown adipose tissue; WAT, white adipose tissue.

of **sarcoplasmic/endoplasmic reticulum Ca^{2+} transport ATPase (SERCA)** is involved in heat production in skeletal muscle [8–10]. Even though, SLN was discovered more than 30 years ago [10,11], the physiological implications of SLN–SERCA interaction are only now beginning to emerge. Therefore, a major objective of this review is to highlight the role of SLN in skeletal muscle physiology, with a focus on thermogenesis and metabolism.

Muscle as a Thermogenic Organ – A Historic Account

The role of skeletal muscle in thermogenesis is well known ever since the earliest biological studies. Muscle shivering is recruited as the first line of defense during an acute exposure to cold

Glossary

Adipokines: peptide hormones secreted from fat tissues.
Brown adipose tissue (BAT): is a highly specialized organ enriched with mitochondria that express the mitochondrial transmembrane protein uncoupling protein 1 (UCP1).
Dysferlinopathies: neuromuscular disorders that are caused by a deficiency of the muscle repair protein dysferlin.
Excitation–contraction coupling: the process in which the action potential generated by motor neurons causes the muscle to undergo contraction.
Facultative diet-induced thermogenesis (fDIT): the increase in energy expenditure in BAT and skeletal muscle in response to overfeeding that helps to protect against weight gain.
Indirect calorimetry: a technique that measures the amount of oxygen inhaled and carbon dioxide exhaled by the body. The volume and ratio of these two gasses helps to determine the metabolic state of the body.
Myokines: they include cytokines or other small peptides secreted by muscle cells that can have autocrine, paracrine or endocrine effects.
Nonshivering thermogenesis (NST): is thermogenic activity that occurs in BAT and muscle that is independent of the repetitive muscular contraction (shivering). The mechanism of activation could be hormonal and/or neuronal.
Obligatory diet-induced thermogenesis (oDIT): is the postprandial increase in energy expenditure above basal metabolism that is required for the digestion, absorption, and storage of nutrients.
PPARγ coactivator 1α (PGC1α): is a transcriptional coactivator that regulates the expression of genes involved in metabolism. It is an important regulator of mitochondrial biogenesis.
Peroxisome proliferator-activated receptor δ (PPARδ): is a nuclear hormone receptor and plays an important role in regulating glucose and lipid metabolism.
Sarcolipin (SLN): is a small protein that coexists with SERCA in the muscle sarcoplasmic reticulum.
Sarcoplasmic reticulum (SR): is a specialized endoplasmic reticulum present in striated muscle cells. It

Table 1. Role of Skeletal Muscle beyond Contraction

Function	Regulator	Study model	Phenotype	Refs
Ca^{2+} handling proteins	SLN	SLN KO mice	Sensitivity to cold-induced thermogenesis and diet-induced obesity (DIO)	[8]
	SLN	Skeletal muscle specific SLN overexpression in mice	Rescue of cold-sensitive phenotype, resistance to DIO, increased fatty acid metabolism and fatigue resistance	[8,53]
	PLB	PLB skeletal muscle overexpression in mice	Centronuclear myopathy like phenotype	[80]
	RYR1	Mice with leaky RYR1	Malignant hyperthermia, increased skeletal muscle metabolism	[39]
Metabolic regulators	PPARδ/β	Treatment with PPARδ agonist in mice	Increased fatty acid oxidation, resistance to HFD-induced obesity in mice	[81]
	PPARα	Skeletal muscle specific PPARα overexpression in mice	Resistance to obesity, glucose intolerance	[82]
	PGC1α	Skeletal muscle specific PGC1α overexpression in mice	Increased oxidative fibers, increased fatigue resistance and endurance capacity	[83,84]
	PGC1α	Skeletal muscle specific PGC1α KO mice	Decreased oxphos genes, abnormal glucose homeostasis	[85,86]
	PGC1β	Skeletal muscle specific PGC1β overexpression in mice	Increased oxidative fibers, increased oxidative work capacity	[87]
	PGC1β	Skeletal muscle specific PGC1β knock out in mice	No effect on glucose homeostasis	[88]
	PGC1α, PGC1β	Skeletal muscle specific double knockout	Decreased exercise performance, decreased oxidative capacity, mitochondrial structural derangements	[88]
	Estrogen-related receptor γ (ERR-γ)	Skeletal muscle specific ERR-γ overexpression in mice	Increased exercise capacity, increased mitochondrial enzyme activity	[89]
	Leptin/ AMP activated kinase (AMPK)	Leptin treatment ± AMPK inhibitor	Decreased mitochondrial respiration when AMPK is inhibited	[90]
	Leptin/ Phosphoinositide 3 kinase (PI3 K)	Leptin treatment ± PI3 K inhibitor	Decreased mitochondrial respiration when PI3 K is inhibited	[90]
	Nur77	Skeletal muscle specific Nur77 overexpression in mice	Increased oxidative metabolism, improved cold tolerance	[91]
	Lipoprotein lipase (LPL)	Skeletal muscle specific LPL overexpression in mice	Increased cold tolerance	[92]

acts as a Ca^{2+} store and regulates Ca^{2+} concentration in the cell.
Sarcoplasmic/endoplasmic reticulum Ca^{2+} ATPase (SERCA): is a P-type calcium ATPase present on SR. It transports calcium from cytoplasm into the SR and hydrolyzes ATP in the process.
Thermogenesis: the process of heat generation in the body.
Uncoupling protein 1 (UCP1): a transmembrane protein present in the mitochondrial membrane of brown adipose tissue (BAT). It uncouples the oxidative phosphorylation from ATP synthesis, generating heat in the process.

Table 1. (continued)

Function	Regulator	Study model	Phenotype	Refs
Mitochondrial dynamics regulators	Mitofusin 2 (Mfn2)	Muscle specific expression analysis	Decreased skeletal muscle Mfn2 expression in obese humans	[93]
	Mfn2	Mfn2 repression in myotubes	Decreased glucose oxidation, decreased respiration	[93]
	Dynamin-related protein 1 (Drp1)	Ceramide induced Drp1 overexpression in myotubes	Decreased mitochondrial respiration, disrupted insulin signaling. Drp1 knockdown prevented these effects	[94]
Myokines	IL-6	Il-6 KO mice	Develop late onset obesity, impaired glucose homeostasis	[95]
	Irisin	Increased FNDC5/Irisin in human myocytes/ adipocyte culture	Increased energy expenditure	[96]
	FGF 21	Muscle specific constitutive activation of mTORC1 in mice	Increased plasma FGF 21, lean mice, increased insulin sensitivity, increased fatty acid oxidation	[79]
Hormones	Thyroid	Thyroid receptor ∝ Knockouts	Muscle LPL and deiodinases are recruited to compensate for reduced BAT function	[97]
	β2-adrenoceptor	β1-, β2-, and β3-adrenoceptor global knockout mice	Enhanced insulin sensitivity, increased adiposity, and glucose intolerance	[98]
	Glucocorticoid	Treatment of rats with dexamethazone	Mitochondrial biogenesis in skeletal muscle	[69]
Ca^{2+} signaling regulators	Ca^{2+}/Calmodulin dependent protein kinase kinase ∝ (CamKK∝)	Increased expression in mice skeletal muscle using vectors	Increased glucose uptake in skeletal muscle	[99]
	Calcineurin	Activated calcineurin in mice skeletal muscle	Increased lipid oxidation, increased glycogen synthesis, increased oxidative phosphorylation, increased expression of PPAR∝ and δ, and PGC1∝	[73]

[12]. Shivering is a repetitive contraction–relaxation process activated by repeated stimulation of the neuromuscular junction that leads to elevation of cytosolic Ca^{2+} concentration, thereby activating ATP hydrolysis to produce heat. During shivering, heat is primarily produced by the major ATP-utilizing enzymes including Na^+/K^+ ATPase, myosin ATPase, and SERCA [12–14]. Interestingly, it has been shown that mice and humans can maintain body temperature during acute cold exposure, even when shivering is minimized [8,15]. Studies have also shown that during cold acclimation, shivering is gradually replaced by **nonshivering thermogenesis (NST)**. This is because repetitive muscle contractions during constant shivering can cause muscle damage [16]. In addition, high-intensity shivering relies predominantly on muscle glycogen that can become limiting after few hours [15].

Many studies using rodent models suggested that **brown adipose tissue (BAT)** is the major site of NST. BAT expresses **uncoupling protein 1 (UCP1)**, which functions to deplete the

proton motive force developed in the mitochondrial intermembranous space by the electron transport pathway, and is the basis for thermogenesis in BAT [17]. However, in large mammals including humans, although BAT is abundant in neonatal stages, it decreases in adulthood [14,18]. The development of UCP1 knockout (KO) animals has revealed the crucial importance of BAT in NST. Cold adaptation studies performed in UCP1 KO mice showed that UCP1-independent thermogenesis exists even in mice, because these mice could be gradually cold-adapted and survive to full term [19]. These mechanisms include but are not limited to futile metabolic cycles like phosphocreatine cycling in beige fat, futile protein turnover, and futile SERCA activity in muscle [8,20–23]. Interestingly, BAT is either absent or inactive in certain endotherms including birds and in few mammals (boars and pigs) [24,25], suggesting that alternate thermogenic mechanisms must exist to maintain a constant body temperature (Tc). The predominant use of mice, which rely on BAT, as experimental animals for thermogenesis studies, has often neglected the importance of skeletal muscle in temperature homeostasis. Initial cold adaptation studies performed in avian species provided evidence for the existence of NST in the skeletal muscle and suggested that the SR Ca^{2+} handling plays an important role in this process [24,26]. It was elegantly shown that oxygen consumption increased in skeletal muscle of cold-acclimated ducklings [27]. Dumonteil *et al.* demonstrated that muscle SERCA activity (measured as Ca^{2+} uptake rate) is significantly higher in cold-acclimated ducklings compared to ducklings maintained at thermoneutrality [26]. More recent studies suggest that skeletal muscle SR Ca^{2+} cycling, specifically uncoupling of SERCA activity by SLN, could be an important mechanism for muscle-based thermogenesis in mammals including mice [8,23,28,29]. There is also evidence that NST mechanisms are activated during many other physiological contexts such as fear, fever, and caloric (diet)-overload [30–32]. However, it is currently not known to what extent NST pathways are recruited during each of these physiological conditions other than cold.

Calcium Cycling and Muscle Heat Production – Early Discoveries

Muscle function is intricately associated with oscillation of Ca^{2+} ion concentration in the cytosol. The role of SR calcium cycling in **excitation–contraction (EC) coupling** is well defined [33], while its influence on metabolic activity of skeletal muscle has not been adequately explored. During muscle contraction, Ca^{2+} cycling is initiated by membrane depolarization, activating dihydropyridine receptor (DHPR), a voltage dependent L-type calcium channel present on T-tubules. These channels can in turn activate the release of Ca^{2+} from SR through ryanodine receptor 1 (RYR1), whose activity is fine-tuned by modulatory proteins like triadin, junctin, and calsequestrin that together form the calcium-releasing unit (CRU) [33]. When the cytosolic Ca^{2+} builds up, it binds to myofilaments and bring about contraction [34]. At the same time, a rise in cytosolic Ca^{2+} can trigger key Ca^{2+} dependent signaling pathways and also activate mitochondrial respiration. Once the cytosolic Ca^{2+} levels exceed the activation threshold of the SERCA, it transports the Ca^{2+} into the SR against the concentration gradient, utilizing energy from ATP hydrolysis thereby initiating relaxation [33]. Two major isoforms of SERCA are expressed in skeletal muscle: SERCA 1a and 2a. SERCA 1a isoform is expressed predominantly in fast glycolytic fibers, while SERCA 2a is predominantly expressed in slow twitch and fast-oxidative fibers. SERCA 2a is the main isoform in cardiac muscle. The SERCA activity in muscle is regulated by two small proteins: phospholamban (PLB) and SLN, which will be discussed in detail below [35]. In addition to SLN and PLB, a new peptide regulator of SERCA, myoregulin, has been identified; however there is paucity of information and its physiological role is less well understood [36]. The first clear evidence that SR calcium cycling by SERCA can be adapted to generate heat comes from studies on deep-sea fishes called blue marlin. These fishes contain a special organ called 'heater organ', that is a modified extraocular muscle tissue [7,37]. The heater organ is enriched with SR and mitochondria to generate heat. The SR is highly specialized with an inherently leaky RyR that increases SERCA pump activity and therefore ATP hydrolysis and heat production. This is combined with enhanced mitochondrial oxidative metabolism to

support ATP production and heat generation [38]. Another example where uncontrolled SR Ca^{2+} release and uptake can result in excessive heat is associated with a pathological condition called 'malignant hyperthermia (MH)' found in pigs and humans [39]. MH is caused by mutations in the RYR1 that become leaky when exposed to volatile anesthetics (even exercise in some cases), causing excessive Ca^{2+} leak from the SR that leads to continuous cycling of SERCA pump and myosin ATPase, thereby generating excessive heat. Increased SERCA activity and SR remodeling has also been shown to be involved during cold adaptation in rabbits, where BAT is limited in amounts [40]. Studies in birds (BAT absent) further demonstrated that RYR1 and SERCA expression were upregulated after cold adaptation, suggesting a role for SR Ca^{2+} handling in thermogenesis [26]. Despite these findings, the detailed mechanism that leads to increased heat production from SERCA activity during cold challenge remained unclear.

Sarcolipin, a Regulator of SERCA Pump, Is Essential for Muscle Thermogenesis and Body Temperature Maintenance

As mentioned above, the activity of SERCA pump in cardiac and skeletal muscle is regulated by PLB and SLN. PLB is a 52-aa phosphoprotein that inhibits SERCA activity at low cytosolic Ca^{2+}. Release of Ca^{2+} from SR and/or phosphorylation of PLB relieve inhibition of SERCA [35,41]. Studies have shown that PLB is an important mediator of β-adrenergic response in the heart and responsible for modulating the force frequency of heart [42]. On the other hand, SLN expression is relatively high in skeletal muscle, but its expression is restricted to atrial chamber in the heart. Compared to PLB, SLN is relatively small (31 aa) and has distinct N- and C-terminal residues [35,43,44]. Recent studies have shown that SLN interaction with SERCA is unique in that it can bind to SERCA in the presence of high cytosolic Ca^{2+} [22,43]. The mechanism behind SLN-mediated muscle-based thermogenesis is not fully understood. However, recent studies have shown that when SLN binds to SERCA, it allows ATP hydrolysis to occur, but the Ca^{2+} transport into the SR is decreased due to 'slippage' of Ca^{2+} back to the cytosol. This suggests that in the presence of SLN, more ATP needs to be hydrolyzed by SERCA to transport the released Ca^{2+} than in the absence of SLN. There is also evidence that pretreatment with the RYR inhibitor dantrolene increases the sensitivity of mice to acute cold exposure [8]. Also, hyperphosphorylation of RyR1 sites, which can make it leaky, is observed in conditions when muscle-based thermogenesis is higher [16,23]. This implies that Ca^{2+} leak from RYR is important for muscle-based thermogenesis. Taken together these data suggest that the mechanism behind SLN-mediated NST is a result of futile SERCA activity leading to an increase in ATP hydrolysis and heat production. Interestingly, no such uncoupling-based mechanism has been reported in MH or heater organ. Those mechanisms rely mostly on futile Ca^{2+} cycling. More recent X-ray crystallographic studies further confirmed SLN–SERCA interaction can occur in the Ca^{2+}-bound state [45].

Although *in vitro* studies suggested that SLN binding to SERCA could increase heat production [22,46–48], its role in muscle thermogenesis remained unclear. Bal *et al.* first studied the importance of SLN in temperature homeostasis using a SLN knockout (SLN KO) mice [8,49]. When challenged to acute cold (4 °C), these mice were unable to maintain Tc [8], providing the first physiological evidence for SLN as a contributor in thermogenesis. Reintroduction of SLN in the SLN KO background, using skeletal-muscle-specific SLN overexpression, rescued the cold-sensitive phenotype. Further, cold exposure studies using curare (a neuromuscular junction inhibitor), and interscapular BAT removal surgery, suggested that SLN is a contributor in muscle-based NST [8]. Interestingly, SLN-mediated NST in muscle is recruited to a greater extent in mice lacking UCP1. In addition, mice lacking both SLN and UCP1 are much more sensitive to exposure to acute cold of 4 °C, than UCP1 KO mice [28]. The role of SLN in muscle-based NST was further evidenced by the recent observation that neonatal mice acclimatized to cold retain higher levels of SLN in skeletal muscle [29].

SLN Plays a Critical Role in Whole Body Energy Metabolism

Studies have shown that humans can produce heat in response to normal feeding, a phenomenon termed thermic effect of food or **obligatory diet-induced thermogenesis** (oDIT) [50]. Interestingly, overfeeding and high-fat diet (HFD) studies have demonstrated that rodents and humans can increase energy expenditure to prevent excessive weight gain. This is known as **facultative diet-induced thermogenesis** (fDIT) and has become an attractive target for combating obesity. [31,51,52]. These studies also highlighted the role of BAT and UCP1 as a major mechanism for fDIT in rodents. However it remains unclear if BAT is an important component of fDIT in humans. The skeletal muscle, being a major metabolic organ, could be involved in fDIT. Since SLN was found to be involved in cold-activated thermogenesis, it is possible that SLN might be recruited in fDIT. Bal *et al.* used mice with either null for SLN (SLN KO) or skeletal-muscle-specific overexpression (SLN OE) to investigate the role of muscle in fDIT, *in vivo*. SLN KO and SLN OE mice were fed on HFD for 12 weeks. SLN KO mice on HFD developed increased adiposity and insulin resistance compared to WT mice, which were less obese but exhibited upregulated SLN expression in selected muscle tissues [8]. Interestingly, SLN OE mice were found to be resistant to HFD-induced obesity and did not exhibit metabolic disease [53]. SLN OE mice on HFD exhibited a higher metabolic rate compared to their controls and their muscles showed enhanced oxidative capacity. This was accompanied by increased mitochondrial biogenesis and fatty acid transport protein expression. These studies highlighted that SLN is recruited in fDIT, and increased SLN level/activity in the skeletal muscle promotes increased fat oxidation and offers protection against diet-induced obesity (DIO). However, details are missing and the mechanism behind fatty-acid-based activation of SLN mediated NST is yet to be understood. Interestingly, mice overexpressing SLN in muscle were able to exercise better and were found to be resistant to fatigue [54]. Collectively these studies suggested that SLN is an important regulator of muscle metabolism.

The Relevance of SLN Expression in Neonatal and Adult Skeletal Muscle

In rodents SLN expression is regulated in a developmental and tissue specific manner. SLN expression is induced in all skeletal muscles during late embryonic development (~1 week before birth) and the level of expression increases right after birth reaching its maximum 1–3 days after birth in rodents [55]. Gradually, SLN expression is down regulated during neonatal development and by the weaning age (~21 days old) it becomes restricted to slow-twitch/oxidative fibers. In adult mice (8–16 weeks old) the relative SLN expression level can be ordered as follows: atria > diaphragm > soleus > tongue > red gastrocnemius > trapezius > oxidative fiber-rich portion of quadriceps > oxidative fiber-rich portion of tibialis anterior. It is interesting that one of the most critical challenges that a newborn mammal faces in the extrauterine environment is triggering thermogenesis to maintain Tc on their own. Importantly, during neonatal stage the muscles are not mature enough to support shivering, neonates lack insulation by fur, and their surface-to-body ratio is higher causing significant heat loss [56]. In spite of the above odds, neonate mice are able to survive because they are equipped with abundant BAT. The presence of relatively high SLN expression in mouse and rat skeletal muscles during the first two weeks after birth suggests that SLN is important for muscle-based NST in neonates that lack shivering ability [29,55]. It was recently demonstrated that when neonatal mice are acclimatized and reared in cold, SLN expression is retained and continues to express at higher levels beyond weaning age, when compared to mice reared at thermoneutrality. Along with SLN, the expression of SERCA 2a isoform is much higher in cold-reared mice [29]. This delay in the isoform switch from SERCA 2a to SERCA 1a, as normally observed during development, has also been shown in cold-acclimated ducklings [26]. As mentioned earlier, SERCA 2a isoform is expressed predominantly in slow twitch/fast oxidative muscle. In addition, SERCA 2a exhibits slower Ca^{2+} transport kinetics than SERCA 1a, possibly due to lower affinity for Ca^{2+} [57,58].

The upregulation of SERCA 2a expression in cold-adapted neonates suggests a shift in the fiber type towards slow oxidative. It is possible that the slow kinetics of SERCA 2a along with interaction with SLN support the heat generating capacity of skeletal muscle. In a recent study Bal *et al.* have shown that conditional ablation of interscapular BAT in adult mice induces SLN expression in muscle, when exposed to cold challenge [23]. These studies provide convincing evidence that SLN–SERCA interaction is an important component of muscle-based thermogenesis. They further suggest that the expression of SLN is regulated by physiological demand.

Significance of Increased SLN Expression in Dystrophic/Diseased Skeletal Muscle

Dystrophic muscle disease has a major effect on muscle structure/function and metabolism. It is often associated with poor muscle function but with increased energy demand [59]. Further, the calcium cycling is altered due to membrane instability and disorganization of SR structure. In Duchenne muscular dystrophy (DMD) the glycolytic muscles are more severely affected and there is an increase in oxidative fiber type [60]. Recent studies have shown that expression of SLN is upregulated severalfold in the mouse models of DMD [61]. In addition, there is an upregulation in protein expression of SERCA 2a in these dystrophic muscles. Few studies have highlighted the impact of DMD on muscle and whole body metabolism [62,63]. In addition to DMD, SLN expression is also upregulated in rodent skeletal muscles in various **dysferlinopathies**, α-tocopherol deficiency, myotonic dystrophy, and dynamin dependent centronuclear myopathy [64–67]. Since metabolic demand is high in these muscles, the upregulation of SLN could be part of a global process promoting the oxidative metabolism to cope with the compromised muscle function.

SLN Promotes Oxidative Metabolism in Skeletal Muscle in Response to Increased Metabolic Demand

DIO, muscle disease, exercise, and cold impose a metabolic demand on the body, which necessitates reallocation of energy (and/or substrate) utilization between different organs. Under such conditions the skeletal muscle shows flexibility in substrate utilization to accommodate the needs of other organs including brain. It is well known that oxidative muscles can cope with greater energy demand and are less vulnerable to fatigue than glycolytic fast twitch muscles [68]. Their machinery is ideally suited to support long enduring physical tasks. During cold exposure, oxidative muscles are better suited to generate heat, because of their ability to maintain high-energy demand. Shifting to oxidative metabolism is also beneficial in redistributing fuel sources and energy consumption between different organs. Conditions of high/altered energy demand impose metabolic stress and trigger the release of stress hormones like glucocorticoids, which are known to activate mitochondrial biogenesis in skeletal muscle [69]. Interestingly, glucocorticoids are also known to upregulate the expression of SLN in skeletal muscle [70]. The presence of SLN in slow twitch/oxidative fibers, in rodents, indicates that there is a physiological connection between SLN expression and oxidative metabolism. The fact that SLN overexpression in fast twitch glycolytic muscle promoted mitochondrial biogenesis and oxidative metabolism further supports this connection [53]. An interesting finding was that SLN overexpression increased **PGC1α** and **PPARδ** expression and resulted in higher calcineurin activity, which are in line with higher mitochondrial biogenesis. These studies also suggested that SLN regulates muscle metabolism by altering cytosolic Ca^{2+} (Figure 2). Specifically, SLN uncoupling of SERCA leads to an elevation in cytosolic Ca^{2+} that serves as a powerful signal (i) to facilitate Ca^{2+} entry into mitochondria and activate mitochondrial metabolism and (ii) to activate Ca^{2+} dependent signaling pathways including calcineurin and Ca^{2+}/calmodulin dependent protein kinase II (CamKII), important for programming the muscle into an oxidative phenotype through the activation of PGC1α and PPARs [71–73].

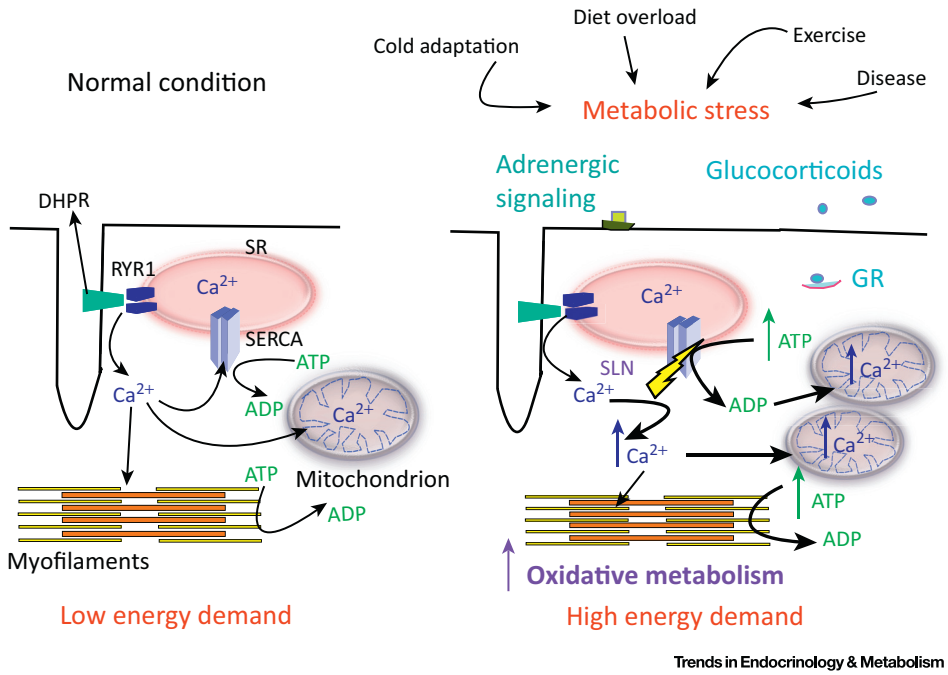

Figure 2. Sarcolipin Promotes Oxidative Metabolism under Conditions of Increased Energy Demand. During muscle contraction, membrane depolarization leads to activation of DHPR/RYR1 complex leading to release of Ca^{2+} from the SR. As the cytosolic Ca^{2+} concentration rises, it binds to myofilaments and triggers muscle contraction. Elevated cytosolic Ca^{2+} activates SERCA which pumps Ca^{2+} back into the SR. The energy demand during normal activity (routine functions) is relatively low. On the other hand, many different pathophysiological states increase energy demand in muscle. In addition, several cytokines and neurohormonal mechanisms (including glucocorticoids and adrenergic signaling) are activated to orchestrate muscle metabolism. SLN expression is also upregulated under these high-energy demand conditions. Higher SLN/SERCA level leads to higher cytosolic Ca^{2+} and facilitates its uptake into mitochondria, which serves as a trigger for oxidative metabolism. In addition Ca^{2+}-signaling pathways activate increased transcription of genes involved in oxidative metabolism. Abbreviations: DHPR, dihydropyridine receptor; GR, glucocorticoid receptor; RYR1, ryanodine receptor 1; SERCA, sarcoplasmic/endoplasmic reticulum calcium ATPase; SLN, sarcolipin; SR, sarcoplasmic reticulum.

Concluding Remarks and Future Perspectives

In the mammalian body, skeletal muscle is one of the most abundant tissues and serves as a major metabolic organ. Because of its ability to generate heat through shivering and non-shivering mechanisms, muscle can play important homeostatic roles, in thermogenesis as well as in whole body metabolism by regulating fuel utilization. Therefore, future research should explore how NST in muscle can be exploited to regulate whole body energy metabolism. Recent discoveries have uncovered the importance of crosstalk between muscle and other organs in the regulation of energy metabolism both under healthy and diseased conditions [51,74]. Muscle also has enormous flexibility in substrate utilization and can switch back and forth between fat and carbohydrate to accommodate energy demand and substrate availability during various pathophysiological conditions. Studies using mouse models have highlighted that SLN-mediated SR Ca^{2+} cycling and hence intracellular Ca^{2+} plays an important role in integrating muscle energy demand with energy production. Although significant progress has been made recently in understanding the function of SLN, much remains to be understood regarding its regulation and mechanism of heat production (see Outstanding Questions). The importance of SLN in human physiology remains relatively unknown. A recent study has shown that SLN-mediated metabolism is downregulated in obese humans [75]. These data are exciting and warrant further studies, to discover the full potential of SLN–SERCA-mediated muscle-based thermogenesis in humans. Determining the factors that can directly regulate SLN's expression and/or function will

Outstanding Questions

How does muscle integrate contractile, thermogenic, and metabolic functions? Is it through Ca^{2+} regulation or **myokines,** or through mechanisms yet to be defined?

During metabolic overload, how does muscle interact with other organs including liver? Is it entirely through myokines or through other mechanisms including metabolites?

How does sarcolipin–sarcoplasmic/endoplasmic reticulum Ca^{2+} ATPase (SLN–SERCA) interaction modify overall Ca^{2+} homeostasis in muscle? What is the impact of increasing SLN on plasma membrane and mitochondrial regulators of Ca^{2+} entry and efflux?

How does SLN interaction with SERCA lead to increased oxidative metabolism? Is prolonged increase in SLN expression beneficial or detrimental?

Does SLN promote mitochondrial biogenesis through known or novel signaling mechanisms?

Is upregulation of SLN beneficial under diseased conditions? What roles do inflammatory pathways play in upregulating SLN expression?

Is there a relationship between myokine production and SLN expression? Is muscle thermogenesis synchronized by autocrine or paracrine action of slow-twitch muscles, where SLN expression is more abundant?

Is SLN–SERCA interaction an ideal target to increase energy metabolism and combat metabolic disorders? Can SLN levels be manipulated pharmacologically?

provide attractive therapeutic targets for enhancing skeletal muscle metabolism and opportunities to counter obesity-related metabolic diseases.

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References

1. Szent-Gyorgyi, A.G. (2004) The early history of the biochemistry of muscle contraction. *J. Gen. Physiol.* 123, 631–641
2. Huxley, H.E. and Hanson, J. (1959) The structural basis of the contraction mechanism in striated muscle. *Ann. N. Y. Acad. Sci.* 81, 403–408
3. Taylor, N.A. and Wilkinson, J.G. (1986) Exercise-induced skeletal muscle growth. Hypertrophy or hyperplasia? *Sports Med.* 3, 190–200
4. Hoppeler, H. (1986) Exercise-induced ultrastructural changes in skeletal muscle. *Int. J. Sports Med.* 7, 187–204
5. Egan, B. and Zierath, J.R. (2013) Exercise metabolism and the molecular regulation of skeletal muscle adaptation. *Cell Metab.* 17, 162–184
6. Zurlo, F. *et al.* (1990) Skeletal muscle metabolism is a major determinant of resting energy expenditure. *J. Clin. Invest.* 86, 1423–1427
7. Block, B.A. (1994) Thermogenesis in muscle. *Annu. Rev. Physiol.* 56, 535–577
8. Bal, N.C. *et al.* (2012) Sarcolipin is a newly identified regulator of muscle-based thermogenesis in mammals. *Nat. Med.* 18, 1575–1579
9. Odermatt, A. *et al.* (1998) Sarcolipin regulates the activity of SERCA1, the fast-twitch skeletal muscle sarcoplasmic reticulum Ca^{2+}-ATPase. *J. Biol. Chem.* 273, 12360–12369
10. Wawrzynow, A. *et al.* (1992) Sarcolipin, the 'proteolipid' of skeletal muscle sarcoplasmic reticulum, is a unique, amphipathic, 31-residue peptide. *Arch. Biochem. Biophys.* 298, 620–623
11. MacLennan, D.H. *et al.* (1973) Isolation of sarcoplasmic reticulum proteins. *Cold Spring Harb. Symp. Quant. Biol.* 37, 469–477
12. Haman, F. (2006) Shivering in the cold: from mechanisms of fuel selection to survival. *J. Appl. Physiol. (1985)* 100, 1702–1708
13. Clarke, R.J. *et al.* (2013) Quantitative calculation of the role of the Na(+),K(+)-ATPase in thermogenesis. *Biochim. Biophys. Acta* 1827, 1205–1212
14. Rowland, L.A. *et al.* (2015) The role of skeletal-muscle-based thermogenic mechanisms in vertebrate endothermy. *Biol. Rev. Camb. Philos. Soc.* 90, 1279–1297
15. Haman, F. *et al.* (2016) Oxidative fuel selection and shivering thermogenesis during a 12- and 24-h cold-survival simulation. *J. Appl. Physiol. (1985)* 120, 640–648
16. Aydin, J. *et al.* (2008) Nonshivering thermogenesis protects against defective calcium handling in muscle. *FASEB J.* 22, 3919–3924
17. Meyer, C.W. *et al.* (2010) Adaptive thermogenesis and thermal conductance in wild-type and UCP1-KO mice. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* 299, R1396–R1406
18. Cypess, A.M. *et al.* (2009) Identification and importance of brown adipose tissue in adult humans. *N. Engl. J. Med.* 360, 1509–1517
19. Ukropec, J. *et al.* (2006) UCP1-independent thermogenesis in white adipose tissue of cold-acclimated $Ucp1^{-/-}$ mice. *J. Biol. Chem.* 281, 31894–31908
20. She, P. *et al.* (2007) Disruption of BCATm in mice leads to increased energy expenditure associated with the activation of a futile protein turnover cycle. *Cell Metab.* 6, 181–194
21. Kazak, L. *et al.* (2015) A creatine-driven substrate cycle enhances energy expenditure and thermogenesis in beige fat. *Cell* 163, 643–655
22. Sahoo, S.K. *et al.* (2013) 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
23. Bal, N.C. *et al.* (2016) Increased reliance on muscle based thermogenesis upon acute minimization of brown adipose tissue function. *J. Biol. Chem.* 291, 17247–17257
24. Bicudo, J.E. *et al.* (2001) Thermogenesis in birds. *Biosci. Rep.* 21, 181–188
25. Berg, F. *et al.* (2006) The uncoupling protein 1 gene (UCP1) is disrupted in the pig lineage: a genetic explanation for poor thermoregulation in piglets. *PLoS Genet.* 2, e129
26. Dumonteil, E. *et al.* (1993) Sarcoplasmic reticulum Ca^{2+}-ATPase and ryanodine receptor in cold-acclimated ducklings and thermogenesis. *Am. J. Physiol.* 265, C507-13
27. Duchamp, C. and Barre, H. (1993) Skeletal muscle as the major site of nonshivering thermogenesis in cold-acclimated ducklings. *Am. J. Physiol.* 265, R1076-83
28. Rowland, L.A. *et al.* (2015) 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
29. Pant, M. *et al.* (2015) Cold adaptation overrides developmental regulation of sarcolipin expression in mice skeletal muscle: SOS for muscle-based thermogenesis? *J. Exp. Biol.* 218, 2321–2325
30. Marks, A. *et al.* (2009) Nonshivering thermogenesis without interscapular brown adipose tissue involvement during conditioned fear in the rat. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* 296, R1239–R1247
31. Tremblay, A. *et al.* (2013) Adaptive thermogenesis can make a difference in the ability of obese individuals to lose body weight. *Int. J. Obes. (Lond).* 37, 759–764
32. Monda, M. *et al.* (1994) Non-shivering thermogenesis during prostaglandin E1 fever in rats: role of the cerebral cortex. *Brain Res.* 651, 148–154
33. Lamb, G.D. (2000) Excitation–contraction coupling in skeletal muscle: comparisons with cardiac muscle. *Clin. Exp. Pharmacol. Physiol.* 27, 216–224
34. Szent-Gyorgyi, A.G. (1975) Calcium regulation of muscle contraction. *Biophys. J.* 15, 707–723
35. Shaikh, S.A. *et al.* (2016) 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
36. Anderson, D.M. *et al.* (2015) A micropeptide encoded by a putative long noncoding RNA regulates muscle performance. *Cell* 160, 595–606
37. da Costa, D.C. and Landeira-Fernandez, A.M. (2009) Thermogenic activity of the Ca^{2+}-ATPase from blue marlin heater organ: regulation by KCl and temperature. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* 297, R1460–R1468

38. Block, B.A. *et al.* (1994) Characterization of the sarcoplasmic reticulum proteins in the thermogenic muscles of fish. *J. Cell Biol.* 127, 1275–1287
39. Robinson, R. *et al.* (2006) Mutations in RYR1 in malignant hyperthermia and central core disease. *Hum. Mutat.* 27, 977–989
40. Arruda, A.P. *et al.* (2008) Cold tolerance in hypothyroid rabbits: role of skeletal muscle mitochondria and sarcoplasmic reticulum Ca^{2+} ATPase isoform 1 heat production. *Endocrinology* 149, 6262–6271
41. James, P. *et al.* (1989) Nature and site of phospholamban regulation of the Ca^{2+} pump of sarcoplasmic reticulum. *Nature* 342, 90–92
42. Koss, K.L. and Kranias, E.G. (1996) Phospholamban: a prominent regulator of myocardial contractility. *Circ. Res.* 79, 1059–1063
43. Sahoo, S.K. *et al.* (2015) 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
44. Odermatt, A. *et al.* (1997) Characterization of the gene encoding human sarcolipin (SLN), a proteolipid associated with SERCA1: absence of structural mutations in five patients with Brody disease. *Genomics* 45, 541–553
45. Winther, A.M. *et al.* (2013) The sarcolipin-bound calcium pump stabilizes calcium sites exposed to the cytoplasm. *Nature* 495, 265–269
46. Smith, W.S. *et al.* (2002) Sarcolipin uncouples hydrolysis of ATP from accumulation of Ca^{2+} by the Ca^{2+}-ATPase of skeletal-muscle sarcoplasmic reticulum. *Biochem. J.* 361, 277–286
47. Meis, L.D. (2000) Energy interconversion by the sarcoplasmic reticulum Ca^{2+}-ATPase: ATP hydrolysis, Ca^{2+} transport, ATP synthesis and heat production. *An. Acad. Bras. Cienc.* 72, 365–379
48. Mall, S. *et al.* (2006) The presence of sarcolipin results in increased heat production by Ca^{2+}-ATPase. *J. Biol. Chem.* 281, 36597–36602
49. Babu, G.J. *et al.* (2007) Ablation of sarcolipin enhances sarcoplasmic reticulum calcium transport and atrial contractility. *Proc. Natl. Acad. Sci. U. S. A.* 104, 17867–17872
50. Westerterp, K.R. (2004) Diet induced thermogenesis. *Nutr. Metab. (Lond).* 1, 5
51. Tseng, Y.H. *et al.* (2010) Cellular bioenergetics as a target for obesity therapy. *Nat. Rev. Drug Discov.* 9, 465–482
52. Rothwell, N.J. and Stock, M.J. (1979) A role for brown adipose tissue in diet-induced thermogenesis. *Nature* 281, 31–35
53. Maurya, S.K. *et al.* (2015) 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
54. Sopariwala, D.H. *et al.* (2015) Sarcolipin overexpression improves muscle energetics and reduces fatigue. *J. Appl. Physiol. (1985)* 118, 1050–1058
55. Babu, G.J. *et al.* (2007) Differential expression of sarcolipin protein during muscle development and cardiac pathophysiology. *J. Mol. Cell Cardiol.* 43, 215–222
56. Asakura, H. (2004) Fetal and neonatal thermoregulation. *J. Nippon Med. Sch.* 71, 360–370
57. Periasamy, M. and Kalyanasundaram, A. (2007) SERCA pump isoforms: their role in calcium transport and disease. *Muscle Nerve* 35, 430–442
58. Brini, M. *et al.* (2012) Calcium pumps: why so many? *Compr. Physiol.* 2, 1045–1060
59. Nowak, K.J. and Davies, K.E. (2004) Duchenne muscular dystrophy and dystrophin: pathogenesis and opportunities for treatment. *EMBO Rep.* 5, 872–876
60. Rafael, J.A. *et al.* (2000) Dystrophin and utrophin influence fiber type composition and post-synaptic membrane structure. *Hum. Mol. Genet.* 9, 1357–1367
61. Schneider, J.S. *et al.* (2013) Increased sarcolipin expression and decreased sarco(endo)plasmic reticulum Ca^{2+} uptake in skeletal muscles of mouse models of Duchenne muscular dystrophy. *J. Muscle Res. Cell Motil.* 34, 349–356
62. Pant, M. *et al.* (2015) Metabolic dysfunction and altered mitochondrial dynamics in the utrophin-dystrophin deficient mouse model of Duchenne muscular dystrophy. *PLoS One* 10, e0123875
63. Percival, J.M. *et al.* (2013) Defects in mitochondrial localization and ATP synthesis in the mdx mouse model of Duchenne muscular dystrophy are not alleviated by PDE5 inhibition. *Hum. Mol. Genet.* 22, 153–167
64. Vasu, V.T. *et al.* (2009) Sarcolipin and ubiquitin carboxy-terminal hydrolase 1 mRNAs are over-expressed in skeletal muscles of α-tocopherol deficient mice. *Free Radic. Res.* 43, 106–116
65. Campanaro, S. *et al.* (2002) Gene expression profiling in dysferlinopathies using a dedicated muscle microarray. *Hum. Mol. Genet.* 11, 3283–3298
66. Liu, N. *et al.* (2011) Mice lacking microRNA 133a develop dynamin 2-dependent centronuclear myopathy. *J. Clin. Invest.* 121, 3258–3268
67. Osborne, R.J. *et al.* (2009) Transcriptional and post-transcriptional impact of toxic RNA in myotonic dystrophy. *Hum. Mol. Genet.* 18, 1471–1481
68. Wang, Y. and Pessin, J.E. (2013) Mechanisms for fiber-type specificity of skeletal muscle atrophy. *Curr. Opin. Clin. Nutr. Metab. Care* 16, 243–250
69. Weber, K. *et al.* (2002) Glucocorticoid hormone stimulates mitochondrial biogenesis specifically in skeletal muscle. *Endocrinology* 143, 177–184
70. Gayan-Ramirez, G. *et al.* (2000) Corticosteroids decrease mRNA levels of SERCA pumps, whereas they increase sarcolipin mRNA in the rat diaphragm. *J. Physiol.* 524, 387–397
71. Wu, H. *et al.* (2002) Regulation of mitochondrial biogenesis in skeletal muscle by CaMK. *Science* 296, 349–352
72. Wright, D.C. *et al.* (2007) Calcium induces increases in peroxisome proliferator-activated receptor γ coactivator-1α and mitochondrial biogenesis by a pathway leading to p38 mitogen-activated protein kinase activation. *J. Biol. Chem.* 282, 18793–18799
73. Long, Y.C. *et al.* (2007) Calcineurin regulates skeletal muscle metabolism via coordinated changes in gene expression. *J. Biol. Chem.* 282, 1607–1614
74. Argiles, J.M. *et al.* (2016) Skeletal muscle regulates metabolism via interorgan crosstalk: roles in health and disease. *J. Am. Med. Dir. Assoc.* Published online June 17, 2016. http://dx.doi.org/10.1016/j.jamda.2016.04.019
75. Paran, C.W. *et al.* (2015) Reduced efficiency of sarcolipin-dependent respiration in myocytes from humans with severe obesity. *Obesity (Silver Spring)* 23, 1440–1449
76. Hood, D.A. *et al.* (2006) Coordination of metabolic plasticity in skeletal muscle. *J. Exp. Biol.* 209, 2265–2275
77. Munoz-Canoves, P. *et al.* (2013) Interleukin-6 myokine signaling in skeletal muscle: a double-edged sword? *FEBS J.* 280, 4131–4148
78. Pedersen, B.K. (2011) Muscles and their myokines. *J. Exp. Biol.* 214, 337–346
79. Guridi, M. *et al.* (2015) Activation of mTORC1 in skeletal muscle regulates whole-body metabolism through FGF21. *Sci. Signal.* 8, ra113
80. Fajardo, V.A. *et al.* (2015) Phospholamban overexpression in mice causes a centronuclear myopathy-like phenotype. *Dis. Model. Mech.* 8, 999–1009
81. Tanaka, T. *et al.* (2003) Activation of peroxisome proliferator-activated receptor δ induces fatty acid β-oxidation in skeletal muscle and attenuates metabolic syndrome. *Proc. Natl. Acad. Sci. U. S. A.* 100, 15924–15929
82. Finck, B.N. *et al.* (2005) A potential link between muscle peroxisome proliferator- activated receptor-α signaling and obesity-related diabetes. *Cell Metab.* 1, 133–144
83. Calvo, J.A. *et al.* (2008) Muscle-specific expression of PPAR(coactivator-1α improves exercise performance and increases peak oxygen uptake. *J. Appl. Physiol. (1985)* 104, 1304–1312
84. Lin, J. *et al.* (2002) Transcriptional co-activator PGC-1α drives the formation of slow-twitch muscle fibres. *Nature* 418, 797–801
85. Handschin, C. *et al.* (2007) Skeletal muscle fiber-type switching, exercise intolerance, and myopathy in PGC-1α muscle-specific knock-out animals. *J. Biol. Chem.* 282, 30014–30021

86. Handschin, C. *et al.* (2007) Abnormal glucose homeostasis in skeletal muscle-specific PGC-1α knockout mice reveals skeletal muscle-pancreatic beta cell crosstalk. *J. Clin. Invest.* 117, 3463–3474

87. Arany, Z. *et al.* (2007) The transcriptional coactivator PGC-1β drives the formation of oxidative type IIX fibers in skeletal muscle. *Cell Metab.* 5, 35–46

88. Zechner, C. *et al.* (2010) Total skeletal muscle PGC-1 deficiency uncouples mitochondrial derangements from fiber type determination and insulin sensitivity. *Cell Metab.* 12, 633–642

89. Rangwala, S.M. *et al.* (2010) Estrogen-related receptor γ is a key regulator of muscle mitochondrial activity and oxidative capacity. *J. Biol. Chem.* 285, 22619–22629

90. Solinas, G. *et al.* (2004) The direct effect of leptin on skeletal muscle thermogenesis is mediated by substrate cycling between *de novo* lipogenesis and lipid oxidation. *FEBS Lett.* 577, 539–544

91. Chao, L.C. *et al.* (2012) Skeletal muscle Nur77 expression enhances oxidative metabolism and substrate utilization. *J. Lipid. Res.* 53, 2610–2619

92. Jensen, D.R. *et al.* (2008) Increased thermoregulation in cold-exposed transgenic mice overexpressing lipoprotein lipase in skeletal muscle: an avian phenotype? *J. Lipid. Res.* 49, 870–879

93. Bach, D. *et al.* (2003) Mitofusin-2 determines mitochondrial network architecture and mitochondrial metabolism. A novel regulatory mechanism altered in obesity. *J. Biol. Chem.* 278, 17190–17197

94. Smith, M.E. *et al.* (2013) Mitochondrial fission mediates ceramide-induced metabolic disruption in skeletal muscle. *Biochem. J.* 456, 427–439

95. Wallenius, V. *et al.* (2002) Interleukin-6-deficient mice develop mature-onset obesity. *Nat. Med.* 8, 75–79

96. Huh, J.Y. *et al.* (2014) Irisin stimulates muscle growth-related genes and regulates adipocyte differentiation and metabolism in humans. *Int. J. Obes. (Lond).* 38, 1538–1544

97. Pelletier, P. *et al.* (2008) Mice lacking the thyroid hormone receptor-α gene spend more energy in thermogenesis, burn more fat, and are less sensitive to high-fat diet-induced obesity. *Endocrinology* 149, 6471–6486

98. Asensio, C. *et al.* (2005) The lack of β-adrenoceptors results in enhanced insulin sensitivity in mice exhibiting increased adiposity and glucose intolerance. *Diabetes* 54, 3490–3495

99. Hinkley, J.M. *et al.* (2014) Constitutively active CaMKKα stimulates skeletal muscle glucose uptake in insulin-resistant mice *in vivo*. *Diabetes* 63, 142–151