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Sarcolipin is a novel regulator of muscle metabolism and obesity

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Abstract

Obesity is increasing at an alarming rate, both in adults and adolescents, across the globe due to increased consumption of caloric rich diet. Obesity and its associated complications appear to be major contributing factors not only to diabetes/heart disease but also to cancer, and neurological diseases causing a huge burden on the health care system. To date, there are no effective treatments to reduce weight gain, other than caloric restriction and exercise which are often difficult to enforce. There are very few drugs available for treating obesity and those that are available only reduce obesity by ~10%. Identifying mechanisms to increase energy expenditure, on top of the increase elicited by exercise, would be more beneficial to control weight gain. The purpose of this review is to highlight the role of sarcolipin (SLN), a regulator of SERCA pump, in muscle thermogenesis and metabolism. We will further discuss if enhancing SLN activity could be an effective mechanism to increase energy expenditure and control weight gain. We will also discuss the merits of adaptive thermogenesis in muscle and brown fat as potential mechanisms to increase energy expenditure during caloric overload. That said, there is still a great need for further research into the mechanism of diet induced thermogenesis and its relevance to overall metabolism and obesity.

Graphical Abstract

Sarcolipin increases energy cost

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Keywords

Obesity; Muscle metabolism; Sarcolipin; SERCA; Thermogenesis; Energy expenditure

Introduction

Obesity in humans is a consequence of overeating which can cause a chronic imbalance between caloric intake and energy expenditure. Exercise has been proven as an effective strategy to increase energy expenditure and maintain weight loss but it is often difficult to adhere in individuals who are obese. Therefore alternative methods to enhance energy expenditure in muscle should be explored. Enhancing energy expenditure by means other than exercise including activation of nonshivering thermogenesis that contribute to increased energy expenditure or pathways that decrease metabolic efficiency can become effective to counter rate of obesity (weight gain) and metabolic disorders. One such mechanism is activation of adaptive thermogenesis in Brown adipose tissue (BAT). Several studies in rodents have shown that BAT mediated thermogenesis can be recruited to increase energy expenditure [1–6] in response to high caloric diet such as high fat diet. However the significance of BAT mediated thermogenesis is less clear in humans since it is relatively a minor component in adult humans and current research is aimed at activating BAT tissue in humans [7–10].

On the other hand skeletal muscle represents ~ 40 % of body mass and is a major consumer of metabolites [11]. Therefore, skeletal muscle is an ideal organ that can be exploited to increase energy expenditure [12–15]. More recent studies highlight that skeletal muscle is an important player in adaptive thermogenesis and it is actively recruited during cold adaptation, expending significant amount of energy [16, 17]. Existing data also suggest that skeletal muscle could be an important component in diet induced thermogenesis [16, 18–23]. However the mechanism(s) regulating muscle energy expenditure independent of muscle contraction is less well understood. Recent studies suggest that maintenance of Ca^{2+} ion gradient by the SERCA pump in resting muscle can be a significant energy consuming process [24]. The purpose of this review is to discuss the role of Sarcolipin (SLN), a novel regulator of SERCA pump in metabolism and thermogenesis. Recent studies suggest that SLN binding to SERCA promotes uncoupling of SERCA pump from Ca^{2+} transport

resulting in futile cycling of SERCA, increased ATP hydrolysis and heat production in muscle [25, 26]. Here we will discuss the findings from SLN^{-/-} and SLN overexpression (SLN^{OE}) mouse models which further highlight the importance of SLN based thermogenesis in cold adaptation and as well during diet overload [14, 16, 27, 28]. Lastly we will discuss the merits of activating adaptive thermogenesis especially in muscle to increase energy expenditure and manage weight gain.

Sarcoplasmic reticulum is a critical player in muscle physiology

The sarcoplasmic reticulum (SR) is a highly specialized membrane network and a Ca²⁺ storage system in skeletal and cardiac muscle. This intracellular membrane network maintains a high Ca²⁺ gradient in the SR lumen and facilitates Ca²⁺ release and uptake during contractile activity. The sarco-endoplasmic reticulum (SR) Ca²⁺ ATPase (SERCA) is a Ca²⁺ ion transport pump and is primarily responsible for SR Ca²⁺ refilling and causing muscle relaxation [29]. In skeletal muscle both SERCA1 and SERCA2 isoforms are present; the heart muscle primarily expresses SERCA2a [30]. Regulation of SERCA activity in muscle plays a fundamental role in Ca²⁺ homeostasis as it maintains both the resting Ca²⁺ concentration and the SR Ca²⁺ load needed for muscle contraction [29, 31]. The SERCA pump activity in muscle is regulated by two small proteins: phospholamban (PLB) and sarcolipin (SLN). The role of PLB has been extensively studied in cardiac muscle physiology and it is known as a force frequency modulator of heart function and is the principle mediator of β -adrenergic response of the heart [32, 33]. On the other hand the physiological and functional significance of SLN is an emerging area of research. SLN is a small protein (SLN) consisting of 31 amino acids long and is exclusively expressed in striated muscle; it is expressed at higher levels in skeletal muscle and to a lesser extent in cardiac atria [34]. SLN expression is also developmentally regulated in rodents; it is relatively high in fetal and neonatal skeletal muscles but gradually becomes restricted to slow-twitch oxidative muscle fibers in adult rodents [34]. The expression of SLN is independently regulated from SERCA and can be affected by disease states [35, 36]. The expression of SLN varies in small and large mammals. Compared to rodents, SLN is expressed several fold higher in both fast twitch and slow twitch muscles of adult larger mammals including rabbits, dogs and humans [37]. The role of SLN in muscle physiology remained unclear until recently. Some studies even suggested that it may be very similar to PLB in function [38]. However SLN expression is quite distinct from PLB; SLN is highly abundant in fast and slow/oxidative muscles of large mammals including human [34].

SR Ca²⁺ cycling plays a role in muscle thermogenesis

Skeletal muscle has long been known as a major site of thermogenesis. Shivering of skeletal muscle is a well-established mechanism of heat production where repetitive contraction results in generation of heat. However, prolonged shivering is detrimental to muscle function and survival of the organism, therefore needs to be replaced with nonshivering mechanisms of heat production. Nonshivering thermogenesis (NST) has been shown to occur mainly in brown adipose tissue (BAT). BAT is an important organ highly specialized to generate heat through nonshivering thermogenesis and it is relatively abundant in neonatal stages of most mammals and in rodents. However in adult stages of large mammals

it is either absent or a minor component (as found in humans) and therefore large mammals have to rely on muscle for their adaptive thermogenesis. Several studies have shown that skeletal muscle also plays an important role in nonshivering thermogenesis [39–41] and especially, the sarcoplasmic reticulum (SR) Ca^{2+} -transport might be an important component in nonshivering thermogenesis [42]. The importance of SR Ca^{2+} -ATPase (SERCA) in heat generation was initially demonstrated in a group of endothermic open-ocean billfishes called blue marlin (of the genus *Makaira*) [43, 44]. In these fishes, a part of their ocular muscle (located inside their cranial cavity) is modified into a specialized tissue that produces heat upon exposure to cold. This tissue termed as “heater organ” has allowed billfishes to maintain cranial temperature $\sim 15^{\circ}\text{C}$ higher than the ambient water temperature. The heater organ is filled with SR membranous network surrounded by many mitochondria; continuous leak and reuptake of Ca^{2+} by SERCA leads to heat generation [42–46]. The involvement Ca^{2+} cycling in heat production is also observed in a pathological condition called “malignant hyperthermia (MH)”, caused by a mutation of the ryanodine receptor that results in uncontrolled Ca^{2+} leak from the SR activating SERCA and heat production [47, 48]. Muscle-based Ca^{2+} cycling in thermogenesis was suggested in cold acclimatized birds [49–51]. In mammals, the importance of SERCA in heat production was suggested in cold acclimatized rabbits by Arruda *et. al.* [52–54]. Slow-twitch skeletal muscles of cold-acclimatized mice showed about two-fold more heat production. However the mechanism(s) that contribute to NST in skeletal muscle has been less well understood.

SLN uncouples SERCA pump activity, increasing ATP hydrolysis and heat production

SLN is only 31 amino acids long and co localizes with SERCA in muscle SR. The mechanism of SLN interaction with SERCA has been recently studied by several groups including our laboratory [25, 55–58]. Recent crystallographic and protein cross linking studies have shown that monomeric SLN binds to the transmembrane groove of SERCA in the SR in a Ca^{2+} dependent manner [56, 57]. Interestingly the characteristics of SLN binding to SERCA are quite different from PLB. It is known that PLB can only bind to Ca^{2+} free SERCA and its binding lowers SERCA pump affinity for Ca^{2+} but PLN inhibition can be relieved by high Ca^{2+} or phosphorylation of the protein [32]. On the other hand SLN can bind to Ca^{2+} bound SERCA and this can be seen even at high Ca^{2+} [25, 55, 58]. SLN does not affect pump affinity for Ca^{2+} but reduces the V_{max} of Ca^{2+} uptake. Interestingly, SLN can remain bound to SERCA during Ca^{2+} transport and causes uncoupling of Ca^{2+} transport from ATP hydrolysis [16, 25, 55]. By this mechanism SLN promotes futile cycling of the pump consuming more ATP for Ca^{2+} transport. These *in vitro* studies suggested that SLN can uncouple SERCA pump ATP hydrolysis from Ca^{2+} transport; promoting futile cycling of SERCA at the expense of increased ATP hydrolysis and contributing to increased heat production in muscle [25, 26, 55, 59, 60]. Based on these findings it was speculated that SLN interaction with SERCA could contribute to thermogenesis and whole body temperature homeostasis.

To address the role of SLN in thermogenesis, our laboratory developed a SLN knockout (*Sln*^{−/−}) mouse model [61]. By challenging these mice to acute cold (4°C), Bal *et. al.* showed

that SLN is indispensable for the maintenance of core body temperature [16]. Although *Sln*^{-/-} mice were able to maintain their whole body temperature at 22 °C, when exposed to acute cold 4 °C, the *Sln*-deficient mice (interscapular BAT was surgically removed: iBAT ablated) failed to maintain whole body TC whereas, iBAT ablated WT mice were able to maintain optimal TC (iBAT ablation minimizes contribution from BAT), suggesting that SLN is able to compensate for loss of BAT function. In addition the cold sensitive phenotype of *Sln*-knockout mice could be rescued by muscle specific SLN overexpression (*Sln*^{OE}). More recently Rowland *et. al.* showed that mice without functional BAT (UCP1 null) or SLN can be gradually cold adapted and cold adaptation upregulates SLN in UCP1 null mice and *vice versa* suggesting that in rodents they might play compensatory roles [17]. These studies clearly show that SLN regulation of SERCA activity is critical for muscle heat production [62].

The role of SLN in muscle metabolism and energy expenditure

Given the close ties between thermogenesis and metabolism, the role of SLN in diet induced thermogenesis has also been investigated in *Sln*^{-/-} mice using HFD feeding [16, 23, 28]. Interestingly, these studies found that loss of SLN in mice resulted with a robust obesity phenotype compared to WT mice- which is evidenced by increased body weight and adiposity (Figure. 1). This obesity phenotype of *Sln*-knockout mice was associated with poor glucose tolerance, a sign of cardiometabolic disease development [16, 23]. In contrast, wild-type mice on HFD were less obese but markedly induced sarcolipin expression levels in skeletal muscle. Remarkably, the obesity phenotype of *Sln*^{-/-} mice was distinguished even in the presence of intact brown adipose tissue. It is likely that the absence of SLN leads to reduced energy expenditure and more efficient energy storage leading to greater fat accumulation. These exciting findings implicated that SLN/SERCA interaction hence Ca²⁺ cycling in muscle is a key component of thermogenesis and can affect whole body energy balance.

SLN is an important component of Basal Metabolic Rate

The role of SLN in energy expenditure was studied using a mouse model (*Sln*^{OE}) that overexpresses SLN (under the control of muscle skeletal α -actin promoter) in all skeletal muscle tissues [14, 27]. SLN overexpression in mice did not adversely affect muscle function but increased the exercise capacity suggesting that SLN could be beneficial to muscle function [27]. Interestingly, *Sln*^{OE} mice was found to consume more calories compared to *Sln*^{-/-} and WT mice when fed *ad libitum*. This raised a fundamental question; whether SLN is a component of basal metabolic rate (BMR). To address this question, used a pair feeding strategy was adapted to investigate the metabolic differences between *Sln*^{-/-} and *Sln*^{OE} mice [14]. In this study, *Sln*^{-/-} and *Sln*^{OE} mice (along with WT) were given identical amount of food for a period of 9 weeks. Interestingly pair-feeding resulted in loss of body weight and depletion of fat depots in *Sln*^{OE} mice as compared to WT and *Sln*^{-/-} mice. This outcome suggested that increasing the SLN expression levels in muscle can lead to higher energy cost for muscle work since there was no difference in physical activity level between groups. Notably, these studies were done under thermoneutrality (to minimize contribution from cold induced thermogenic mechanisms); therefore, the higher energy

expenditure seen here could be attributed to SLN mediated energy expenditure. These findings suggest that a higher SLN to SERCA ratio is energetically costly for routine muscle activity. Based on these studies it can be inferred that SLN could be an important component of BMR.

Higher SLN expression level provides resistance against diet-induced obesity

Loss of SLN induced obesity when fed on HFD, and this prompted us to investigate if higher SLN level could be beneficial and protect mice from diet induced obesity. Interestingly *Sln*^{OE} mice fed on HFD for 12 weeks were quiet resistant to gain weight and were significantly less obese than their WT counterparts [14]. The obesity resistant phenotype in *Sln*^{OE} mice was observed despite the higher caloric consumption than WT controls. Further *Sln*^{OE} mice did not exhibit metabolic abnormalities including hyperglycemia, glucose intolerance and hyperlipidemia. Energy expenditure of *Sln*^{OE} mice was significantly higher which was amplified during night time (active phase of mice). Higher oxygen consumption was also observed in isolated muscle samples consistent with in vivo data. In addition the *Sln*^{OE} mice showed higher fat utilization as evidenced by lower RER values (closer to 0.7). These data suggested that SLN and muscle based adaptive thermogenesis can be recruited during diet overload to increase energy expenditure.

Mechanism behind SLN based adaptive thermogenesis

The *Sln*^{OE} mice fed on HFD showed a striking increase in mitochondrial content, especially in fast glycolytic skeletal muscles like tibialis anterior (TA) muscle (Figure. 2) and extensor digitorum longus (EDL) [14]. The mitochondria in *Sln*^{OE} muscle were much larger with intricate and densely packed cristae and the fast twitch muscle showed higher oxidative capacity in contrast to mitochondria of *Sln*^{-/-} muscle that showed loosely packed cristae. Interestingly, this increase in oxidative phenotype of skeletal muscle occurred independent of fiber type switching since myosin isoforms were not altered. Additionally SLN overexpression increases the expression of key transcriptional regulators (PPAR δ , PGC1 α and NRFs) involved in mitochondrial biogenesis and several genes involved in fatty acid oxidation. However it remains unclear if SLN is directly responsible for this mitochondrial phenotype or this is a consequence of HFD feeding and activation of SLN based thermogenesis. Despite these interesting findings, the detailed mechanisms(s) how SLN affects muscle metabolism is not that well understood. It is well known that beta adrenergic activation plays a central role in BAT mediated thermogenesis, but there is limited data to show that similar mechanism could activate muscle based thermogenesis. . At this stage, more work is needed to completely establish the interplay between SLN, Ca²⁺ signaling and transcriptional regulation of mitochondrial biogenesis. A simple working model is proposed in Figure 3 to explain how SLN signals to increase muscle metabolism. Increasing SLN activity promotes futile cycling of SERCA pump, increases ATP hydrolysis thereby creating a chronic energy demand. Furthermore the uncoupling actions on SERCA increases cytosolic Ca²⁺ allowing Ca²⁺ entry into mitochondria [63–70] and at the same time activating Ca²⁺-dependent signaling pathways promoting mitochondrial biogenesis [71–75].

Conclusions and Perspectives

During the past three decades research on adaptive thermogenesis has been focused on BAT due to the extensive use of mouse as an experimental model. BAT is present and active in both neonatal and adult stages in rodent species. However, BAT is either absent or negligible in adult large mammals including humans [7–9]. The limited presence of active BAT in adult stages questions whether BAT functions primarily as thermogenic organ during neonatal growth or if it is also involved in energy balance and body weight regulation in adult stages. Several studies both in rats and mice have shown that BAT and uncoupling protein 1 (UCP1) is involved in DIT in rodents [1, 4, 76–80]. Although the activation of BAT thermogenesis during diet overload is well demonstrated in rodents; it remains underexplored in humans. BAT has been shown to be activated by cold exposure but not by sympathomimetic agents thus its role in DIT needs further experimentation in humans [81]. The role of BAT in DIT in humans is currently under intense investigation with the discovery of inducible BAT or beige cells upon cold exposure [2, 4, 82–85].

On the other hand skeletal muscle has an enormous capacity to increase metabolic rate due to its sheer abundance; it can be recruited to increase energy expenditure either via exercise and or through a combination of exercise and therapeutics. Further the discovery of SLN provides a novel target to manipulate energy expenditure and whole body metabolism. The recent findings using both sarcolipin null (*Sln*^{−/−}) and sarcolipin overexpression (*Sln*^{OE}) mice highlight that SLN is a key component of muscle energy expenditure and heat production. Studies with *Sln*^{OE} mice also suggested that increasing SLN activity in muscle can be effective method to burn excess calories during HFD. Thus targeting SLN based non-shivering thermogenesis (NST) in muscle could be an effective therapeutic strategy to control weight gain and treat obesity.

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Figure 1. Loss of SLN leads to diet-induced obesity (DIO) whereas overexpression of SLN provides resistance against DIO

Figure 2.
Overexpression of SLN increases oxidative capacity and mitochondrial biogenesis

Figure 3.

Proposed model to show how SLN signals to enhance muscle metabolism (Adapted from Maurya et. al. JBC: 2015)