Cell. Mol. Life Sci. 65 (2008) 3725 – 3736
1420-682X/08/233725-12
DOI 10.1007/s00018-008-8236-6

Cellular and Molecular Life Sciences

Review

Pleiotropic effects of sphingolipids in skeletal muscle

P. Bruni* **and C. Donati**

Dipartimento di Scienze Biochimiche, Università di Firenze, and Istituto Interuniversitario di Miologia (IIM), 50134 Firenze (Italy), Fax: +39 055 459 8905, email: paola.bruni@unifi.it

Received 30 April 2008; received after revision 19 June 2008; accepted 14 July 2008
Online First 1 August 2008

Abstract. Studies of the last two decades have demonstrated that sphingolipids are important signalling molecules exerting key roles in the control of fundamental biological processes including proliferation, differentiation, motility and survival. Here we review the role of bioactive sphingolipids such as ceramide, sphingosine, sphingosine 1-phosphate, ganglioside GM3, in the regulation of skeletal muscle biology. The emerging picture is in favour of a complex role of these molecules, which appear implicated in the activation of muscle resident stem cells, their proliferation and differentiation, finalized at skeletal muscle regeneration. Moreover, they are involved in the regulation of contractile properties, tissue responsiveness to insulin and muscle fiber trophism. Hopefully, this article will provide a framework for future investigation into the field, aimed at establishing whether altered sphingolipid metabolism is implicated in the onset of skeletal muscle diseases and identifying new pharmacological targets for the therapy of multiple illnesses, including muscular dystrophies and diabetes.

Keywords. Skeletal muscle, sphingolipids, sphingosine 1-phosphate, insulin responsiveness, muscle regeneration.

Introduction

It is presently well-accepted that sphingolipids are subjected to a complex and intense metabolism which ensures not only the proper supramolecular organization of biological membranes but also the control of a number of bioactive metabolites capable of regulating cellular, tissue, and systemic vital functions. Moreover, studies performed in the last few years have demonstrated that deregulation of sphingolipid metabolism is implicated in the pathogenesis of important diseases such as atherosclerosis, cancer, inflammation and immune disorders. In this regard it is important to mention that very promising results contributing to the treatment of malignant and immune disorders have been obtained recently by employing drugs and monoclonal antibodies targeted to sphingolipid metabolism (for a recent review see Zeidan and Hannun [1]).

During these years abundant experimental evidence favours a key role for sphingolipids also in the regulation of various biological processes in skeletal muscle. To set the stage for a detailed discussion of the pleiotropic action of sphingolipids in skeletal muscle we will first provide a brief overview of skeletal muscle cell biology as well as sphingolipid metabolism and signalling.

* Corresponding author.

Skeletal muscle structure and cell biology

Adult skeletal muscle is the most abundant tissue of the human body representing approximately half of total body mass. Contraction of skeletal muscle induces coordinated body movements through the direct attachment of individual muscles to the skeleton. A skeletal muscle is composed of many bundles of myofibers which are endowed with contractile properties and constitute the tissue functional units. A single myofiber originates from the fusion of many myoblasts and therefore is multinucleated. Each myofiber contains many myofibrils, which are composed by repeating sarcomeres. Calcium-dependent interaction between sarcomere proteins, actin and myosin, causes reciprocal sliding of the two molecules, contraction of the myofibers and thereby the whole skeletal muscle. Skeletal muscles vary considerably in size, shape and arrangement of myofibers; moreover they often contain a mixture of three different types of myofibers which vary for the myosin isoforms expressed and the consequential contractile characteristics [2]. Type 1 myofibers are slow twitch and fatigue resistant, type 2A myofibers are fast twitch and moderately fatigue resistant and type 2B myofibers are fast twitch and not fatigue resistant. The percentage of individual types of myofibers within skeletal muscles is not fixed and can change throughout life.

Under normal biological conditions adult skeletal muscle is an extremely stable tissue. However, upon damage due to specific diseases, trauma or strong physical exercise, skeletal muscle exhibits a remarkable capacity of self-repair. Regeneration of skeletal muscle almost exclusively depends on tissue-resident stem cells, called satellite cells, which become activated upon skeletal muscle injury, giving rise to proliferating myogenic precursor cells that eventually differentiate and fuse to form multinucleated myotubes [3, 4]. The molecular events that take place subsequent to the activation of satellite cells and allow muscle repair are quite complex and, at present, a matter of intense investigation, also for their critical implication in the development of therapies for many diseases characterized by degeneration of skeletal muscle.

Overview of sphingolipid metabolism

Sphingolipids are ubiquitous constituents of eukaryotic cells, originally considered to have a predominantly structural role as components of lipid bilayers. They have received increased attention in the past two decades after the disclosure of important additional functions in signalling events and regulation of fundamental biological processes.

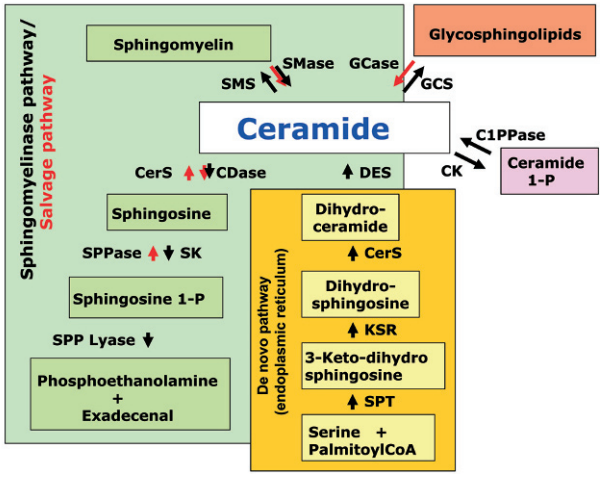

Figure 1. Metabolism of sphingolipids. Ceramide represents the central hub of sphingolipid metabolism. It can be formed by *de novo synthesis*, sphingomyelin hydrolysis and the sphingolipid salvage pathway. Abbreviations: serine palmitoyl-CoA-acyltransferase (SPT), 3-ketosphinganine reductase (KSR), (dihydro)-ceramide synthase (CerS), ceramide desaturase (DES), ceramide kinase (CK), ceramide-1-phosphate phosphatase (C1PPase), glucosylceramide synthase (GCS), glucosyl ceramidase (GCase), ceramidase (CDase), sphingosine-1-phosphate lyase (SPP lyase), sphingosine kinase (SK), sphingosine 1-phosphate phosphatase (SPPase), sphingomyelin synthase (SMS), sphingomyelinase (SMase)

Although a number of issues concerning sphingolipid metabolism remain to be addressed, recently there has been remarkable progress in identifying and cloning the enzymes involved in the *de novo* synthesis of sphingolipids as well as in outlining the route taken by a sphingolipid from initiation of its biosynthesis on the cytosolic leaflet of the endoplasmic reticulum, through its transport from the endoplasmic reticulum to the Golgi apparatus, and its metabolism in the Golgi apparatus. Comprehensive reviews of sphingolipid metabolism have been published recently [5–7]. Here, a brief sketch of the main routes by which sphingolipids are metabolized is provided.

Ceramide, a well-known mediator of various stress responses including cell death and aging [8, 9], occupies a central position in sphingolipid metabolism (Fig. 1). The first step in its *de novo* synthesis involves the formation of 3-keto-dihydrosphingosine through the condensation of serine and palmitoyl-CoA, catalyzed by serine palmitoyl-CoA transferase. In turn, 3-keto-dihydrosphingosine is reduced to dihydrosphingosine, acylated by a ceramide synthase to dihydroceramide which is then converted into ceramide by the action of ceramide desaturase.

Ceramide is a precursor of glycosphingolipids and sphingomyelin, both key components of biological

membranes. In the synthesis of sphingomyelin, a phosphorylcholine group is transferred from phosphatidylcholine to ceramide. Sphingomyelin synthesis occurs in several cellular compartments, although most is synthesized on the lumenal side of the Golgi complex [10, 11]. In vertebrates, glycosphingolipid synthesis is initiated by coupling a glucose or galactose residue in a β-glycosidic linkage to the C1-hydroxyl of ceramide. Specific glycosyltransferases catalyze the transfer of additional single nucleotide-activated sugars onto ceramide, forming more complex glycosphingolipids.

In addition to *de novo* synthesis, ceramide can arise from breakdown of membrane sphingolipids, namely hydrolysis of sphingomyelin, catalyzed by various sphingomyelinases (SMases), differing in their optimal pH and subcellular localization [12], as well as from demolition of glycosphingolipids, to glucosylceramide or galactosylceramide, mediated by specific hydrolases, and the subsequent release of ceramide catalyzed by specific β-glucosidases or galactosidases [13]. Additionally, it must be considered that further metabolism of ceramide gives rise to various metabolites, all endowed with key biological activities. Indeed, ceramide can be phosphorylated to ceramide 1-phosphate by the action of ceramide kinase which, in turn, may be dephosphorylated back to ceramide by a specific phosphatase; alternatively, through the action of a number of ceramidases, ceramide can be deacylated to sphingosine which can be further metabolized to sphingosine-1-phosphate (S1P) by sphingosine kinases (SKs). Intracellular breakdown of S1P relies on the activity of specific phosphatases that generate sphingosine, as well as on S1P lyase which irreversibly cleaves S1P to hexadecenal and ethanolamine phosphate, catalyzing the only exit pathway from sphingolipid metabolism. Finally, it is worth mentioning that sphingosine, which serves as the product of sphingomyelin catabolism, is largely salvaged through reacylation, resulting in the generation of ceramide and its derivatives, in the so-called salvage pathway, contributing to the modulation of ceramide formation [14].

Importantly, in multiple cell types, the metabolites formed by ceramide metabolism, namely ceramide 1-phosphate, sphingosine, S1P, elicit key biological effects, including cell proliferation, differentiation, survival and motility. In this regard, ceramide and sphingosine on the one hand, and S1P on the other, frequently have opposite biological effects. For example, ceramide and sphingosine are generally involved in apoptotic responses to various stress stimuli and in growth arrest, whereas S1P is implicated in mitogenesis, differentiation, and migration [5, 9]. This homeostatic system is frequently referred to as the ceramide/S1P rheostat [15], in which the control of SK activity appears to play a prominent regulatory role [16]. Among the various bioactive sphingolipids, S1P appears to be the only one that exerts its biological action as receptor ligand. In fact, following its intracellular formation, S1P can be released outside the cell through ABCC1-facilitated transport [17], where it can act in a paracrine or autocrine fashion by binding to a panel of specific G-protein coupled receptors originally identified as endothelial differentiation gene receptors and then renamed S1P receptors [18]. The receptor subtypes $S1P_1$, $S1P_2$ and $S1P_3$ are widely expressed, whereas $S1P_4$ and $S1P_5$ are tissue-specific, being expressed almost exclusively in the lymphoid system and in the central nervous system, respectively [18]. Intriguingly, the biological response elicited by S1P in a given cell type appears to be critically dependent on the expression pattern of S1P receptors since they are differentially coupled to heterotrimeric G-proteins and downstream signalling pathways. In this respect, while $S1P_1$ and $S1P_3$ enhance cell motility and migration, $S1P_2$ inhibits chemotactic response [19]. This provides a rationale for the pro-migratory effect exerted by S1P in endothelial cells which exclusively express $S1P_1$ and $S1P_3$ [20], and the chemorepellant action exerted in melanoma cells where $S1P_2$ is the dominant receptor [21].

The pleiotropic role of sphingolipids in the regulation of skeletal muscle cell biology can be fully appreciated by separately examining individual biological effects elicited by components of this lipid class.

Sphingolipids in the regulation of growth, differentiation and regeneration of skeletal muscle

As outlined above, regeneration of skeletal muscle is carried out by satellite cells. These resident stem cells are an heterogeneous population composed of stem cells and committed progenitors. Due to their limited availability and the restricted number of experimental approaches that can be employed to investigate their biological features *in vivo*, myoblastic cell lines, such as the C2C12 which is derived from mouse muscle satellite cells, are widely utilized to study *in vitro* skeletal muscle growth and differentiation. It is presently clear that, upon mitogen depletion, a large subpopulation of C2C12 cells undergoes terminal differentiation and forms myotubes; another relatively small subpopulation called reserve cells remains undifferentiated and shares many characteristics with muscle-resident stem cells, while a third subset of cells undergoes apoptosis [22].

Recent data support a key role of sphingomyelin metabolism and S1P in the activation of satellite cells. By employing lysenin, a protein that specifically binds to surface sphingomyelin, Nagata et al. [23] showed that, in C2C12 reserve cells, which model myogenic cell quiescence [22], as well as in satellite cells, sphingomyelin levels in the plasma membrane of noncycling myogenic cells are high, but then fall as they are activated. Cell membrane sphingomyelin represents a key component of lipid rafts and caveolae, therefore a diminution in sphingomyelin suggests the occurrence of a profound change in the supramolecular organization of plasma membrane of satellite cells, strictly associated with the generation of signals conveying the activation state. This hypothesis is highly plausible in view of the observation that the caveolae-scaffolding protein caveolin-1 is down-regulated in satellite cells during skeletal muscle regeneration [24]. However, the molecular mechanism responsible for the dramatic reorganization of membrane domains that takes place during the transition between the quiescent and the proliferative condition remains to be established. A subsequent study from the same authors [25] has demonstrated that, although the major amount of sphingomyelin is localized in the outer leaflet of plasma membrane, a small pool of sphingomyelin at the inner leaflet is implicated in the signalling of satellite cells, since sphingomyelin levels are maintained when satellite cell activation occurs in the presence of GW4869, inhibitor of neutral SMase. Since in the same report S1P was found to mediate the entry of satellite cells into the cell cycle, the authors concluded that the breakdown of the sphingomyelin pool at the inner leaflet of plasma membrane occurring upon their activation is functional to S1P formation [25]. The potential key role of S1P in skeletal muscle repair was further demonstrated in the same study, since the SK inhibitor dimethylsphingosine severely impaired the regeneration of cardiotoxin-treated skeletal muscle. The involvement of SK/S1P axis in this crucial biological process, however, requires further study given that dimethylsphingosine is rather unspecific, i.e., it has been shown recently to be responsible for suppression of osteoclastogenesis by a mechanism independent of SK inhibition [26]. Nevertheless, the seminal study of Nagata et al. [25] challenges the question of whether the ligation of growth factors and cytokines receptors such as *c-met*, fibroblast growth factor receptor family or CXCR4, which appears to be crucial for satellite cell activation and proliferation [27–29], triggers sphingomyelin hydrolysis in satellite cells and whether this event is implicated in the receptor-mediated biological response.

Pilot studies on the responsiveness of C2C12 myoblasts to challenge with S1P showed that this bioactive lipid triggers numerous signalling pathways by activating phospholipase D [30], monomeric GTPase RhoA [31], and increasing cytosolic calcium [32]. Since S1P action primarily depends on receptor ligation, the expression pattern of S1P receptors was also examined: $S1P_1$, $S1P_2$ and $S1P_3$ were found expressed in C2C12 myoblasts [30]. Intriguingly, the expression of $S1P_2$, which is upstream of phospholipase D activation, was found to be highly diminished throughout myoblast differentiation and substantially abolished in myotubes, suggesting the implication of S1P signalling via $S1P_2$ in the molecular events required to accomplish cell differentiation [33]. Actually, subsequent studies identified S1P as a negative regulator of serum-induced cell proliferation and a powerful activator of myogenic differentiation of C2C12 cells [34]. Notably, although $S1P_1$ and $S1P_3$ are also expressed in these cells, only $S1P_2$ was identified as mediator of S1P biological action. The key role of $S1P_2$ in myogenic differentiation was further strengthened by the observation that ectopic expression of this protein accelerated the onset of a differentiated phenotype. In a subsequent report the S1P-induced regulation of the gap junctional protein connexin-43 has been identified as a downstream event implicated in the promyogenic effect of the sphingolipid [35]. Interestingly, the mechanism by which up-regulation of connexin-43 accounts for the promotion of myogenesis also implicates, besides the exchange of molecules through functional gap-junctions, the specific interaction of the protein with cytoskeleton. In this context it has also been shown that actin remodelling and formation of stress fibers in response to S1P generate a mechanical tension to the plasma membrane of C2C12 cells, activate stretch-activated channels and trigger calcium dependent signals, thus influencing the phenotypic maturation of myoblasts [36].

Experimental evidence has also been recently provided for a physiological role of SK1, one of the two isoforms of S1P-synthesizing enzymes, in the regulation of myoblast proliferation and differentiation. SK1 protein content and S1P formation were found to be enhanced in myoblasts that became confluent as well as in differentiating cells [37]. Moreover, enforced expression of SK1 reduced the myoblast proliferation rate, enhanced the expression of myogenic differentiation markers and anticipated the onset of differentiated muscle phenotype. Conversely, down-regulation of SK1 by specific silencing or overexpression of the catalytically inactive SK1, significantly increased cell growth and delayed the beginning of myogenesis. The biological role exerted by SK1 in myoblasts

appears therefore distinct from that reported in the majority of other cells, where it plays a pivotal role in the promotion of cell proliferation [38]. Furthermore, stimulation of myogenesis in SphK1-overexpressing myoblasts was abrogated when $S1P_2$ was silenced [37], reinforcing the notion that this receptor type is critical for eliciting S1P-mediated effects in these cells. In line with these findings, Donati et al. [39] have shown that the promyogenic effect exerted by low doses of TNFα requires the activation of SK1 and the subsequent engagement of $S1P_2$, demonstrating that the SK1/$S1P_2$ axis is part of the molecular machinery that regulates agonist-dependent myogenesis.

Importantly, the finding that S1P elicits differential effects in satellite cells and myoblasts, promoting proliferation in the former cell type [25] and differentiation in the latter [26], supports the view that the SK/S1P pathway plays a unique role in skeletal muscle regeneration, being required at multiple steps leading skeletal muscle-resident stem cells to repair damaged tissue. This pleiotropic action of S1P likely implies profound changes of signalling pathways downstream of S1PR, the molecular basis of which is largely unknown. A first attempt to identify possible differential S1PR expression between satellite cells and myoblasts, employing C2C12 reserve cells as a model of quiescent satellite cells, did not reveal striking differences, at least at the mRNA level [40]. However, although $S1P_1$ was expressed at the highest level in both cell types, it was found mainly implicated in S1P-regulated cell proliferation in reserve cells, whereas it was uncoupled from DNA synthesis in myoblasts [40]. This supports the view that during the commitment of satellite cells toward myogenic differentiation, critical molecular events occur to readdress the signalling mediated by S1P via its membrane receptors in order to convey distinct biological actions.

A schematic diagram of the effects of sphingolipids on satellite cell activation and myogenic differentiation is illustrated in Figure 2.

Relevant to the role of S1P in skeletal muscle regeneration, it has been reported recently that S1P acts as a powerful stimulator of proliferation of mesoangioblasts [41] which are mesodermic stem cells capable of regenerating skeletal muscle after systemic injection in animal models of muscular dystrophy [42, 43]. Besides exerting a mitogenic effect, S1P was found to robustly protect mesoangioblasts from apoptosis elicited by different stimuli. Notably, the *ex vivo* treatment of mesoangioblasts with the bioactive sphingolipid enhanced their survival after injection into the tibialis anterior muscle of sarcoglycan-null mice, supporting the view that S1P treatment can ameliorate the success of cell therapy aimed at regenerating skeletal muscle.

Cell motility is a key component in the regulation of multiple biological events, including myogenesis. Upon serious skeletal muscle injury, satellite cells from neighbouring intact muscles must be highly motile in order to be recruited at the site of muscle lesion; however, subsequently their cell motility must be negatively regulated in order to establish the proper stable cell-cell contacts and fuse with pre-existing fibers. Interestingly, a recent study reported that along with a promyogenic effect, S1P also exerts anti-migratory action on S1P in C2C12 myoblasts [44]. The lipid mediator reduces the directional cell motility and fully abrogates the chemotactic response to insulin-like growth factor-1. Given the major role of inducer of satellite cell motility exerted by insulin-like growth factor-1 [45], the finding that S1P is capable of fully counteracting its action may have important implications in the *in vivo* regulation of exact satellite cell positioning to cause efficient cell fusion. The anti-migratory response to S1P requires ligation to $S1P_2$, and the subsequent activation of RhoA [44], similar to that observed in other cell systems, such as glioblastoma tumor cells [46]. Intriguingly, it has been observed recently that prostacyclin shares with S1P the unique property of inhibiting the migration of myoblasts [47]. In view of the existing cross-talk between SK and cyclooxygenase signalling pathways in various cell types [48–50], it will be of interest to know whether the anti-migratory action of S1P also involves prostacyclin formation.

Ceramide also appears to be implicated in the regulation of myogenic differentiation. In C2C12 cells the treatment with cell-permeable short chain ceramide suppressed myogenic differentiation, monitored by measuring the activity of the skeletal muscle specific enzyme creatine kinase and the production of insulin-like growth factor 2 [51]. Similarly, in rat myogenic cell line L6 upon induction of the myogenic process by arg8-vasopressin, treatment with medium chain ceramide inhibited the expression of the myogenic marker myogenin [52]. Moreover, during the accomplishment of the myogenic differentiation, ceramide cellular content displayed an early decrease, compatible with the formation of downstream sphingolipids, followed by a marked and sustained increase. Inhibition of ceramide *de novo* synthesis, by fumonisin B1 or myriocin administration, attenuated cellular ceramide levels and, importantly, enhanced the onset of the differentiated phenotype [52] (Fig. 2). In the same study it was proposed that the beneficial effect on myogenesis exerted by the inhibition of ceramide synthesis depends on the negative regulation of phospholipase D brought about by the sphingolipid, being the phosphatidate-dependent signalling pathway required for myogenesis. In support of a negative

role for ceramide in the regulation of myogenesis it has been reported that the inflammatory cytokines TNFα and IL-1β, by stimulating *de novo* ceramide synthesis and by accelerating sphingomyelin breakdown and subsequent ceramide accumulation, antagonize the enhancement of protein synthesis elicited by insulin-like growth factor-1, which parallels the agonist-induced promotion of myogenic phenotype [53]. This finding is in line with the observed negative regulation of translation regulators by ceramide in L6 rat myoblasts [54].

To summarize, the results obtained so far support the view that, in muscle progenitor cells, the relative levels of ceramide and S1P, acting as components of the so-called sphingolipid rheostat, regulate commitment toward myogenic differentiation rather than cell survival as it occurs in different cellular settings [55]. In contrast, very little is known about the role of glycosphingolipids in myogenesis. Pioneering studies indeed showed that myoblast differentiation was paralleled by increased biosynthesis of lactosylceramide and ganglioside GM3 [56, 57]. More recently, cytosolic sialidase Neu2 was reported to be upregulated during C2C12 myoblast differentiation and, importantly, its overexpression was found to enhance myogenesis [58]. Although ganglioside GM3 could represent a major substrate for this enzyme, it remains to be addressed whether the promyogenic effect of sialidase Neu2 depends on the variation of GM3 or lactosylceramide levels. However, in view of the relevance of this issue, the involvement of glycosphingolipids in myoblast differentiation is worthy of detailed investigation.

Effect of sphingolipids on excitation-contraction coupling

The first hint of a role for sphingolipid in the regulation of skeletal muscle physiology came from studies in which it was demonstrated that sphingolipid metabolites could influence muscle contractility by regulating the excitation-contraction coupling. This key process relies on the conformational modification of dihydropyridine receptor induced by plasma membrane depolarization, which by physical interaction opens ryanodine-sensitive Ca^{2+} release channels provoking efflux of Ca^{2+} from the stores of the sarcoplasmic reticulum and activation of the contractile machinery. Among the sphingolipid molecules, sphingosine, but not ceramide, appears to act as a negative modulator of sarcoplasmic reticulum calcium release, acting directly on the ryanodine receptor by inhibiting labelled ryanodine binding to its high affinity site [59]. In favour of a physiological role exerted by sphingosine in skeletal muscle, neutral SMase, which, by initiating sphingomyelin breakdown, is upstream of sphingosine formation, has been identified in junctional T tubule membranes [60]. In agreement, high sphingosine content characterizes skeletal muscle membrane fractions isolated from junctional T tubule, being in principle sufficient for the negative modulation of calcium release *in vivo* [61]. Since many endogenous positive regulators of ryanodine-sensitive calcium channel may constantly act to challenge Ca^{2+} release during the relaxed state, whereas only a restricted number of negative modulators of ryanodine receptor are known, a model has been proposed in which in normal circumstances sphingosine has a major role in maintaining the channel in the closed configuration, until the excitation-contraction coupling is initiated [62]. Conversely, the change in electric field after depolarization is envisioned to be responsible for a diminution of the cationic amphiphile in the microenvironment comprising the ryanodine receptor, favouring its opening. An independent study performed on isolated cut fibers of mouse extensor digitorum longus showed that S1P can also affect the excitation-contraction coupling [63]. Submicromolar concentrations of the bioactive lipid modulates intramembrane charge movements by shifting the voltage threshold, the voltage transition of components of charge, and the inward calcium current through the dihydropyridine receptor. On the basis of the ablation of the effect by suramin treatment, the action of S1P was proposed to implicate engagement of $S1P_3$, which was identified in extensor digitorum longus muscle by RT-PCR.

Effect of sphingolipids on muscle fatigue

Muscles that are intensively used show a progressive decline of performance which largely recovers after a period of rest. This reversible phenomenon is denoted as muscle fatigue. Multiple factors are involved in the onset of fatigue with the relative importance of each dependent on the fiber type composition of the contracting muscles(s), and the intensity, type, and duration of the contractile activity. Major hypotheses of causes of fatigue center on disturbances in the surface membrane and excitation-contraction coupling. Noticeably, sphingosine and S1P, recognized as modulators of excitation-contraction coupling [61, 63], were recently found capable of significantly reducing the tension decline during fatigue of extensor digitorum longus muscle [64]. Interestingly, the slowing of fatigue induced by sphingosine was fully prevented in the presence of dimethylsphingosine, suggesting that its mechanism of action implicates its

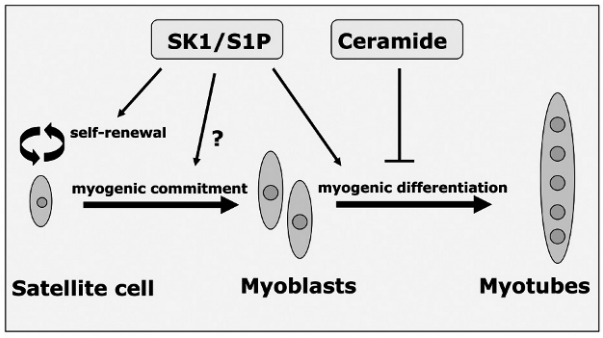

Figure 2. Role of sphingolipids in the activation of satellite cells and myogenic differentiation.

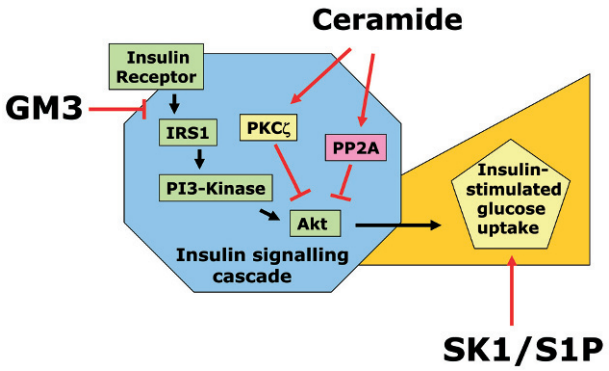

Figure 3. Role of sphingolipids in insulin responsiveness in skeletal muscle.

transformation into S1P. Moreover, the beneficial effect of S1P on fatigue development was still detectable in Ca^{2+}-free solutions, whereas sphingosine was inefficacious unless extracellular Ca^{2+} was available, further supporting the view that sphingosine must be phosphorylated by calcium-dependent SK in order to attenuate the development of fatigue. In myoblasts sphingosine was previously found to bring about activation of phospholipase D via its conversion into S1P [65]. In the same vein, it has been more recently shown that sphingosine could mimic the trophic action of S1P in denervated soleus muscle [66]. Thus, it appears that sphingosine and S1P, which exert opposite biological effects in many other cell systems, mediate similar biological effects in skeletal muscle, likely due to a sufficiently basal active SK, as observed in myoblasts [65], or due to signal-regulated SK activity, as shown in fatigued muscle [64]. The development of fatigue induced by repeated brief contractions provoked a two-fold increase of endogenous content of sphingosine [64]. This finding is in agreement with the significant decrease in ceramide and rise in sphingosine content induced by prolonged exercise in rat skeletal muscle [67].

In view of the important role of fatigue in influencing the performance of skeletal muscle, pharmacological intervention aimed at reducing ceramide accumulation and favouring S1P formation could be exploited in the future to ameliorate skeletal muscle functioning.

All these results support the notion that activation of the whole sphingomyelin hydrolysis pathway plays a key role in the regulation of skeletal muscle contractile properties. With the notable exception of SMase activation [61], however, the complete pattern of enzymes involved still awaits identification.

Effect of sphingolipids on insulin responsiveness of skeletal muscle

The sensitivity of skeletal muscle to insulin is critical for removing excess glucose from the bloodstream after a meal, contributing 80 % of whole body insulin-stimulated glucose disposal. For this reason, insulin resistance in this tissue is generally thought to contribute most significantly to the glucose intolerance associated with nutrient or glucocorticoid oversupply. Obesity exerts a major impact on insulin resistance and is closely associated with type-2 diabetes [68]. This has led to the hypothesis that excessive accumulation of lipids in non-adipose tissues such as skeletal muscle promotes the build-up of metabolites that stimulate negative feed-back pathways to inhibit insulin signalling [69]. Although elevated intramyocellular triacylglycerol levels have been identified as a potential link between increased non-esterified fatty acid availability and insulin resistance, an increasing body of evidence support a key role for ceramide.

Thus far, ceramide has been shown to be enhanced in insulin-resistant muscles in both rodents [70, 71] and humans [72, 73]. Moreover, very recently, increased levels of ceramide have been detected in men at risk of developing type-2 diabetes [74]. In agreement, ceramide has also been shown to induce insulin resistance in a variety of cultured cells [75, 76]. Conversely, exercise training, which improves insulin sensitivity, markedly decreases muscle ceramide levels in both rats and humans [67, 77, 78]. In accordance, interventions capable of improving insulin sensitivity such as administration of troglitazone, agonist of the peroxisome proliferator activated receptor-γ, markedly reduce muscle ceramide levels [79].

Regarding the mechanisms implicated in the action of ceramide, this sphingolipid has been shown to acutely inhibit insulin-stimulated glucose uptake and glycogen synthesis in cultured skeletal muscle cells [75]. A

number of studies have clarified that these effects are due to ceramide-mediated inhibition of several steps of insulin signalling, among which phosphatidylinositol 3-kinase (PI3K)/Akt activation appears to be prominent, downstream of IRS1 phosphorylation [80]. However, the mechanism by which ceramide brings about the inhibition of this key kinase appears to be complex, relying on at least two distinct molecular mechanisms such as Akt dephosphorylation via protein phosphatase 2A-catalyzed reaction [81] and protein kinase Cζ-dependent phosphorylation of Akt's PH-domain, blocking Akt translocation to the membrane [82] (Fig. 3).

In favour of the hypothesis that ceramide acts as a key effector in the induction of insulin resistance in response to enhanced circulating lipids, in C2C12 myotubes palmitate, by activating *de novo* synthesis, increases ceramide content, thereby inhibiting downstream insulin-stimulated Akt phosphorylation [83]. Moreover, the blockade of the *de novo* ceramide synthesis in palmitate-treated C2C12 myotubes relieves the inhibitory effect of saturated non esterified fatty acids toward Akt activation by insulin, while the induction of ceramide accumulation augments the inhibitory effect of saturated non esterified fatty acids [84]. More recently, a study performed in human myoblasts has shown that the employment of myriocin and fumonisin B1 to specifically inhibit distinct enzymes involved in the biosynthesis of ceramide from palmitate, prevented palmitate-induced insulin resistance, further supporting the concept that intramyocellular triacylglycerol levels do not account *per se* for the loss of skeletal muscle responsiveness to insulin [85]. Notably, a study performed using pharmacological agents as well as genetically modified animals to impair ceramide biosynthesis has very elegantly proved that ceramide is a common molecular intermediate linking several different pathological metabolic stresses (i.e., glucocorticoids and saturated fats) to the induction of insulin resistance [86]. Additionally, manipulating ceramide levels in obese rodents markedly attenuates insulin resistance and prevents the onset of diabetes, pointing to a therapeutic relevance for the enzymes of ceramide biosynthetic route [86].

It is also worth mentioning that palmitate-induced ceramide accumulation was found to account for the apoptosis elicited by palmitate in L6 myotubes [87]. Treatment with the ceramide synthase inhibitor fumonisin B1 abrogated the pro-apoptotic effect of palmitate, clearly implicating the *de novo* ceramide synthesis in the process. Interestingly, the inhibition of insulin-stimulated glucose uptake by palmitate administration was efficiently counteracted by blocking caspase-3, clearly demonstrating a relationship between ceramide-mediated apoptosis and insulin resistance in skeletal muscle.

Importantly, it has been shown recently that the SK1/S1P axis is also crucially implicated in the regulation of glucose metabolism in skeletal muscle. In fact, pharmacological or siRNA-mediated inhibition of SK1 resulted in an appreciable decrease in basal and insulin-stimulated glucose uptake in C2C12 myoblasts [88]. Moreover, overexpression of SK1, which caused an increase in extracellular S1P levels, similarly to exogenous S1P enhanced basal and insulin-stimulated glucose uptake. In the same study, SK was found to be involved in insulin signalling, since the enzymatic activity was augmented dose dependently by the hormone. In keeping with its insulin-mimetic effect, SK1 gene delivery was able to reduce blood glucose in a mouse model of type-2 diabetes [88].

Taking into account that SK1 acts as an essential checkpoint in regulating the relative levels of ceramide/sphingosine and S1P [15], in view of the potential therapeutic interest of these findings, it will be important to establish in the future whether the biological effects observed following ectopic SK1 expression in myoblasts are mechanistically linked exclusively to the signalling triggered by S1P or rather implicate also the diminution of ceramide cellular content, similarly to that observed in other cellular settings [89].

A key role on insulin signalling *in vivo* has been demonstrated also for the ganglioside GM3, a molecular species of the glycosphingolipid family that contains a trisaccharide composed of glucose, galactose, and sialic acid linked to ceramide. Employing mutant mice lacking GM3 synthase, which catalyzes the transfer of sialic acid to lactosylceramide and thereby generates GM3, it has been shown that this ganglioside is a negative regulator of insulin signalling and its ablation protects mice from high-fat diet-induced insulin resistance [90]. The increased sensitivity to insulin displayed by the GM3-synthase knock-out mice was due to the enhancement of insulin receptor phosphorylation in skeletal muscle, leading to the hypothesis that the interaction between insulin receptor and GM3 within the glycosphingolipid-enriched membrane domains negatively regulates insulin signalling. The overall role of sphingolipids on insulin responsiveness in skeletal muscle is depicted in Figure 3. Interestingly, the beneficial effect on insulin sensitivity exerted by GM3 synthase knock-out has been shown recently to be mimicked by *in vivo* treatment of Zucker diabetic fatty rats with Genz-123346, inhibitor of glucosylceramide synthase, which catalyzes the first step in the biosynthesis of gangliosides [91]. Although the identity of the critical glycosphingolipid(s) was not directly addressed, in-

triguingly, GM3 content, which was enhanced in Zucker diabetic fatty animals compared to their lean controls, was restored to control levels by pharmacological treatment. Also in this experimental setting, improved glucose control and increased insulin sensitivity were accompanied by an enhanced level of tyrosine phosphorylation of the insulin receptor from the skeletal muscle, in keeping with the hypothesis that glycosphingolipids play a key role in the regulation of insulin signalling in this tissue.

Trophic effect of sphingolipids on skeletal muscle

Skeletal muscle is characterized by a high degree of plasticity. When a muscle remains in disuse for a long period, the rate of degradation of contractile proteins becomes greater than the rate of replacement, resulting in muscle atrophy. This defect may occur as a result of lack of nutrition, loss of nerve supply, micro-gravity, ageing, systemic disease, prolonged immobilization or disuse. Conversely, exercise and nutrition favour hypertrophy, in which the rate of synthesis is much higher than the rate of degradation of contractile proteins, leading to an increase in the size of muscle due to enlargement of existing cell fibers. This unique property permits skeletal muscle adaptation to distinct metabolic and physiological needs. Very interestingly, in a recent study it has been demonstrated that exogenous application of S1P counteracts the reduction of muscle mass caused by denervation, whereas neutralization of the extracellular lipid with a specific anti-S1P antibody accelerates the atrophy caused by denervation [66]. Despite its protective effect on the progression of atrophy, S1P does not affect the extent of denervation-induced apoptosis of skeletal muscle nuclei, indicating a specific trophic action of the sphingolipid, distinct from a basic pro-survival effect. In this regard, the trophic effect of S1P was found to imply the regulation of myosin heavy chain isoform expression, resulting in the attenuation of the slow-to-fast transformation due to inactivity.

Further experimental evidence in favour of a positive role of the SK/S1P pathway in skeletal muscle plasticity comes from a recent report in which global gene expression in skeletal muscle of young men following short-term creatine monohydrate supplementation was examined. SK1 was identified among the most significantly upregulated genes [92], suggesting that enhanced S1P formation is implicated in the increase in fat-free mass and muscle fiber size, which accounts for the beneficial effect of creatine monohydrate on muscle [93, 94]. Since creatine monohydrate also regulates osmosensing properties of muscle, it will be of interest to precisely dissect the molecular events downstream of SK1 responsible for the biochemical and physiological effects of this diet supplement.

Concluding remarks

In this review we have summarised recent advances, to our knowledge, in understanding the role of sphingolipids in the regulation of various aspects of skeletal muscle cell biology. The emerging picture is in favour of a complex action exerted by bioactive sphingolipid metabolites in the control of key processes ranging from skeletal muscle regeneration and plasticity, to contractile properties and insulin-regulated metabolism. However, much remains to be done in order to obtain a clear-cut depiction of the molecular events that participate in the control of sphingolipid metabolism in this tissue. Indeed, in many circumstances it is not known which of the various isoenzymes of sphingolipid metabolism are specifically expressed in skeletal muscle and how their enzymatic activities are interconnected with the signalling network in these cells. Impairment of basic biological processes, such as muscle regeneration or insulin responsiveness, is causative of severe diseases such as muscular dystrophies or type-2 diabetes. We are confident that future investigation in the fascinating field of sphingolipids, addressed to better understand their physiological roles in skeletal muscle, will also individuate new pharmacological targets for the therapy of these diseases.

Acknowledgements. This work was supported in part by funds from the University of Florence and Fondazione Cassa di Risparmio di Pistoia e Pescia.

1 Zeidan, Y. H., Hannun, Y. A. (2007) Translational aspects of sphingolipid metabolism. Trends. Mol. Med. 13, 327–336.
2 Wigmore, P. M., Evans, D. J. (2002) Molecular and cellular mechanisms involved in the generation of fiber diversity during myogenesis. Int. Rev. Cytol. 216, 175–232.
3 Dhawan, J., Rando, T. A. (2005) Stem cells in postnatal myogenesis: molecular mechanisms of satellite cell quiescence, activation and replenishment. Trends Cell. Biol. 15, 666–673.
4 Le Grand, F., Rudnicki, M. A. (2007) Skeletal muscle satellite cells and adult myogenesis. Curr. Opin. Cell. Biol. 19, 628–33.
5 Lahiri, S., Futerman, A. H. (2007) The metabolism and function of sphingolipids and glycosphingolipids. Cell. Mol. Life. Sci. 64, 2270–2284.
6 Zheng, W., Kollmeyer, J., Symolon, H., Momin, A., Munter, E., Wang, E., Kelly, S., Allegood, J. C., Liu, Y., Peng, Q., Ramaraju, H., Sullards, M. C., Cabot, M., Merrill, A. H. Jr. (2006) Ceramides and other bioactive sphingolipid backbones in health and disease: lipidomic analysis, metabolism and roles in membrane structure, dynamics, signalling and autophagy. Biochim. Biophys. Acta. 1758, 1864–1884.
7 Morales, A., Lee, H., Goñi, F. M., Kolesnick, R., Fernandez-Checa, J.C. (2007) Sphingolipids and cell death. Apoptosis. 12, 923–939.

8 Venable, M. E., Lee, J. Y., Smyth, M. J., Bielawska, A., Obeid, L. M. (1995) Role of ceramide in cellular senescence. J. Biol. Chem. 270, 30701–30708.
9 Hannun, Y. A., Luberto, C. (2000) Ceramide in the eukaryotic stress response. Trends Cell. Biol. 10, 73–80.
10 Futerman, A. H., Stieger, B., Hubbard, A. L. and Pagano, R. E. (1990) Sphingomyelin synthesis in rat liver occurs predominantly at the cis and medial cisternae of the Golgi apparatus. J. Biol. Chem. 265, 8650–8657.
11 Jeckel, D., Karrenbauer, A., Birk, R., Schmidt, R. R. and Wieland, F. (1990) Sphingomyelin is synthesized in the cis Golgi. FEBS Lett. 261, 155–157.
12 Marchesini, N., Hannun, Y.A. (2004) Acid and neutral sphingomyelinases: roles and mechanisms of regulation. Biochem. Cell. Biol. 82, 27–44.
13 Valaperta, R., Chigorno, V., Basso, L., Prinetti, A., Bresciani, R., Preti, A., Miyagi, T., Sonnino, S. (2006) Plasma membrane production of ceramide from ganglioside GM3 in human fibroblasts. FASEB J. 20, 1227–1229.
14 Kitatani, K., Idkowiak-Baldys, J., Hannun, Y. A. (2008) The sphingolipid salvage pathway in ceramide metabolism and signalling. Cell. Signal. 20, 1010–1018.
15 Cuvillier, O., Pirianov, G., Kleuser, B., Vanek, P. G., Coso, O. A., Gutkind, S., Spiegel, S. (1996) Suppression of ceramide-mediated programmed cell death by sphingosine-1-phosphate. Nature. 381, 800–803.
16 Gomez-Brouchet, A., Pchejetski, D., Brizuela, L., Garcia, V., Altié, M. F., Maddelein, M. L., Delisle, M. B., Cuvillier, O. (2007) Critical role for sphingosine kinase-1 in regulating survival of neuroblastoma cells exposed to amyloid-beta peptide. Mol. Pharmacol. 72, 341–349.
17 Mitra, P., Oskeritzian, C. A., Payne, S. G., Beaven, M. A., Milstien, S., Spiegel, S. (2006) Role of ABCC1 in export of sphingosine-1-phosphate from mast cells. Proc. Natl. Acad. Sci. U S A. 103, 16394–16399.
18 Birgbauer E, Chun J. (2006) New developments in the biological functions of lysophospholipids. Cell Mol Life Sci. 63, 2695–2701.
19 Donati C, Bruni P. (2006) Sphingosine 1-phosphate regulates cytoskeleton dynamics: implications in its biological response. Biochim. Biophys. Acta. 1758, 2037–2048.
20 Lee M. J., Thangada S, Claffey K. P, Ancellin N, Liu C. H., Kluk M, Volpi M, Sha'afi RI, Hla T. (1999) Vascular endothelial cell adherens junction assembly and morphogenesis induced by sphingosine-1-phosphate.Cell. 99, 301–312.
21 Arikawa K, Takuwa N, Yamaguchi H, Sugimoto N, Kitayama J, Nagawa H, Takehara K, Takuwa Y. (2003) Ligand-dependent inhibition of B16 melanoma cell migration and invasion via endogenous S1P2 G protein-coupled receptor. Requirement of inhibition of cellular RAC activity. J. Biol. Chem. 278, 32841–32851.
22 Yoshida, N., Yoshida, S., Koishi, K., Masuda, K., Nabeshima, Y. (1998) Cell heterogeneity upon myogenic differentiation: down-regulation of MyoD and Myf-5 generates 'reserve cells'. J. Cell. Sci. 111, 769–779.
23 Nagata, Y., Kobayashi, H., Umeda, M., Ohta, N., Kawashima, S., Zammit, P. S., Matsuda, R. (2006) Sphingomyelin levels in the plasma membrane correlate with the activation state of muscle satellite cells. J. Histochem. Cytochem. 54, 375–384.
24 Volonte, D., Liu, Y., Galbiati, F. (2005) The modulation of caveolin-1 expression controls satellite cell activation during muscle repair. FASEB J. 19, 237–239.
25 Nagata, Y., Partridge, T. A., Matsuda, R., Zammit, P.S. (2006) Entry of muscle satellite cells into the cell cycle requires sphingolipid signalling. J. Cell. Biol. 174, 245–253.
26 Kim, H.. J., Lee, Y., Chang, E. J., Kim, H. M., Hong, S. P., Lee, Z. H., Ryu, J., Kim, H. H. (2007) Suppression of osteoclastogenesis by N,N-dimethyl-D-erythro-sphingosine: a sphingosine kinase inhibition-independent action. Mol. Pharmacol. 72, 418–428.
27 Tatsumi, R., Anderson, J. E., Nevoret, C. J., Halevy, O., Allen, R. E. (1998) HGF/SF is present in normal adult skeletal muscle and is capable of activating satellite cells. Dev. Biol. 194, 114–128.
28 Kästner, S., Elias, M. C., Rivera, A. J., Yablonka-Reuveni, Z. (2000) Gene expression patterns of the fibroblast growth factors and their receptors during myogenesis of rat satellite cells. J. Histochem- Cytochem. 48, 1079–1096.
29 Ratajczak, M. Z., Majka, M., Kucia, M., Drukala, J., Pietrzkowski, Z., Peiper, S., Janowska-Wieczorek, A. (2003) Expression of functional CXCR4 by muscle satellite cells and secretion of SDF-1 by muscle-derived fibroblasts is associated with the presence of both muscle progenitors in bone marrow and hematopoietic stem/progenitor cells in muscles. Stem Cells. 21, 363–371.
30 Meacci, E., Vasta, V., Donati, C., Farnararo, M., Bruni, P. (1999) Receptor-mediated activation of phospholipase D by sphingosine 1-phosphate in skeletal muscle C2C12 cells. A role for protein kinase C. FEBS Lett. 457, 184–188.
31 Meacci, E., Donati, C., Cencetti, F., Romiti, E., Bruni, P. (2000) Permissive role of protein kinase C alpha but not protein kinase C delta in sphingosine 1-phosphate-induced Rho A activation in C2C12 myoblasts. FEBS Lett. 482, 97–101.
32 Meacci, E., Cencetti, F., Formigli, L., Squecco, R., Donati, C., Tiribilli, B., Quercioli, F., Zecchi Orlandini, S., Francini, F., Bruni, P. (2002) Sphingosine 1-phosphate evokes calcium signals in C2C12 myoblasts via Edg3 and Edg5 receptors. Biochem. J. 362, 349–357.
33 Meacci, E., Cencetti, F., Donati, C., Nuti, F., Farnararo, M., Kohno, T., Igarashi, Y., Bruni, P. (2003) Down-regulation of EDG5/S1P2 during myogenic differentiation results in the specific uncoupling of sphingosine 1-phosphate signalling to phospholipase D. Biochim. Biophys. Acta. 1633, 133–142.
34 Donati, C., Meacci, E., Nuti, F., Becciolini, L., Farnararo, M., Bruni, P. (2005) Sphingosine 1-phosphate regulates myogenic differentiation: a major role for S1P2 receptor. FASEB J. 19, 449–451.
35 Squecco, R., Sassoli, C., Nuti, F., Martinesi, M., Chellini, F., Nosi, D., Zecchi-Orlandini, S., Francini, F., Formigli, L., Meacci, E. (2006) Sphingosine 1-phosphate induces myoblast differentiation through Cx43 protein expression: a role for a gap junction-dependent and -independent function. Mol. Biol. Cell. 17, 4896–4910.
36 Formigli, L., Meacci, E., Sassoli, C., Squecco, R., Nosi, D., Chellini, F., Naro, F., Francini, F., Zecchi-Orlandini, S. (2007) Cytoskeleton/stretch-activated ion channel interaction regulates myogenic differentiation of skeletal myoblasts. J. Cell. Physiol. 211, 296–306.
37 Meacci, E., Nuti, F., Donati, C., Cencetti, F., Farnararo, M., Bruni, P. (2008) Sphingosine kinase activity is required for myogenic differentiation of C2C12 myoblasts. J. Cell. Physiol. 214, 210–220.
38 Olivera, A., Kohama, T., Edsall, L., Nava, V., Cuvillier, O., Poulton, S., Spiegel, S. (1999) Sphingosine kinase expression increases intracellular sphingosine-1-phosphate and promotes cell growth and survival. J. Cell. Biol. 147, 545–558.
39 Donati, C., Nincheri, P., Cencetti, F., Rapizzi, E., Farnararo, M., Bruni, P. (2007) Tumor necrosis factor-alpha exerts pro-myogenic action in C2C12 myoblasts via sphingosine kinase/S1P2 signaling. FEBS Lett. 581, 4384–4388.
40 Rapizzi, E., Donati, C., Cencetti, F., Nincheri, P., Bruni, P. (2008) Sphingosine 1-phosphate differentially regulates proliferation of C2C12 reserve cells and myoblasts. Mol. Cell. Biochem. 314, 193–199.
41 Donati, C., Cencetti, F., Nincheri, P., Bernacchioni, C., Brunelli, S., Clementi, E., Cossu, G., Bruni, P. (2007) Sphingosine 1-phosphate mediates proliferation and survival of mesoangioblasts. Stem Cells. 25, 1713–1719.
42 Sampaolesi, M., Torrente, Y., Innocenzi, A., Tonlorenzi, R., D'Antona, G., Pellegrino, M. A., Barresi, R., Bresolin, N., De Angelis, M.G., Campbell, K.P., Bottinelli, R., Cossu, G. (2003) Cell therapy of alpha-sarcoglycan null dystrophic mice through intra-arterial delivery of mesoangioblasts. Science. 301, 487–492.

43 Sampaolesi, M., Blot, S., D'Antona, G., Granger, N., Tonlorenzi, R., Innocenzi, A., Mognol, P., Thibaud, J. L., Galvez, B. G., Barthélémy, I., Perani, L., Mantero, S., Guttinger, M., Pansarasa, O., Rinaldi, C., Cusella De Angelis, M. G., Torrente, Y., Bordignon, C., Bottinelli, R., Cossu, G. (2006) Mesoangioblast stem cells ameliorate muscle function in dystrophic dogs. Nature. 444, 574–579.

44 Becciolini, L., Meacci, E., Donati, C., Cencetti, F., Rapizzi, E., Bruni, P. (2006) Sphingosine 1-phosphate inhibits cell migration in C2C12 myoblasts. Biochim. Biophys. Acta. 1761, 43–51.

45 Suzuki, J., Yamazaki, Y., Li, G., Kaziro, Y., Koide, H. (2000) Involvement of Ras and Ral in chemotactic migration of skeletal myoblasts. Mol. Cell. Biol. 20, 4658–4665.

46 Lepley, D., Paik, J.H., Hla, T., Ferrer, F. (2005) The G protein-coupled receptor S1P2 regulates Rho/Rho kinase pathway to inhibit tumor cell migration. Cancer Res. 65, 3788–3795.

47 Bondesen, B. A., Jones, K. A., Glasgow, W. C., Pavlath, G. K. (2007) Inhibition of myoblast migration by prostacyclin is associated with enhanced cell fusion. FASEB J. 21, 3338–3345.

48 Pettus, B. J., Bielawski, J., Porcelli, A. M., Reames, D. L., Johnson, K. R., Morrow, J., Chalfant, C. E., Obeid, L. M., Hannun, Y. A. (2003) The sphingosine kinase 1/sphingosine-1-phosphate pathway mediates COX-2 induction and PGE2 production in response to TNF-alpha. FASEB J. 17, 1411–1421.

49 Ikeda, Y., Ohashi, K., Shibata, R., Pimentel, D. R., Kihara, S., Ouchi, N., Walsh, K. (2008) Cyclooxygenase-2 induction by adiponectin is regulated by a sphingosine kinase-1 dependent mechanism in cardiac myocytes. FEBS Lett. 582, 1147–1150.

50 Johann, A. M., Weigert, A., Eberhardt, W., Kuhn, A. M., Barra, V., Von Knethen, A., Pfeilschifter, J. M., Brüne, B. (2008) Apoptotic cell-derived sphingosine-1-phosphate promotes HuR-dependent cyclooxygenase-2 mRNA stabilization and protein expression. J. Immunol. 180, 1239–1248.

51 Meadows, K. A., Holly, J. M-, Stewart, C.E. (2000) Tumor necrosis factor-alpha-induced apoptosis is associated with suppression of insulin-like growth factor binding protein-5 secretion in differentiating murine skeletal myoblasts. J. Cell. Physiol. 183, 330–337.

52 Mebarek, S., Komati, H., Naro, F., Zeiller, C., Alvisi, M., Lagarde, M., Prigent, A. F., Némoz, G. (2007) Inhibition of de novo ceramide synthesis upregulates phospholipase D and enhances myogenic differentiation. J. Cell. Sci. 120, 407–416.

53 Strle, K., Broussard, S. R., McCusker, R. H., Shen, W. H., Johnson, R. W., Freund, G. G-, Dantzer, R., Kelley, K. W. (2004) Proinflammatory cytokine impairment of insulin-like growth factor I-induced protein synthesis in skeletal muscle myoblasts requires ceramide. Endocrinology. 145, 4592–4602.

54 Hyde, R., Hajduch, E., Powell, D. J., Taylor, P. M., Hundal, H. S. (2005) Ceramide down-regulates System A amino acid transport and protein synthesis in rat skeletal muscle cells. FASEB J. 19, 461–463.

55 Hait, N. C., Oskeritzian, C. A., Paugh, S. W., Milstien, S., Spiegel, S. (2006) Sphingosine kinases, sphingosine 1-phosphate, apoptosis and diseases. Biochim. Biophys. Acta. 1758, 2016–2026.

56 Leskawa, K. C., Hogan, E. L. (1990) Regulation of glycolipid synthesis during differentiation of clonal murine muscle cells. Mol Cell Biochem. 96, 163–173.

57 Cambron, L. D., Leskawa, K. C. (1994) Glycosphingolipids during skeletal muscle cell differentiation: comparison of normal and fusion-defective myoblasts. Mol. Cell. Biochem. 130, 173–185.

58 Fanzani, A., Giuliani, R., Colombo, F., Zizzoli, D., Presta, M., Preti, A., Marchesini, S. (2003) Overexpression of cytosolic sialidase Neu2 induces myoblast differentiation in C2C12 cells. FEBS Lett. 547, 183–188.

59 Betto, R., Teresi, A., Fachechi-Cassano, G., Salviati, G., Sabbadini, R. A. (1992) Sphingosine inhibits calcium release from sarcoplasmic reticulum membranes. Adv. Exp. Med. Biol. 311, 403–404.

60 Ghosh, N., Sabbadini, R., Chatterjee, S. (1998) Identification, partial purification, and localization of a neutral sphingomyelinase in rabbit skeletal muscle: neutral sphingomyelinase in skeletal muscle. Mol. Cell. Biochem. 189, 161–168.

61 Sabbadini R, McNutt W, Jenkins G, Betto R, Salviati G. (1993) Sphingosine is endogenous to cardiac and skeletal muscle. Biochem. Biophys. Res. Commun. 193, 752–728.

62 Sabbadini, R. A., Danieli-Betto, D., Betto, R. (1999) The role of sphingolipids in the control of skeletal muscle function: a review. Ital. J. Neurol. Sci. 20, 423–430.

63 Bencini, C., Squecco, R., Piperio, C., Formigli, L., Meacci, E., Nosi, D., Tiribilli, B., Vassalli, M., Quercioli, F., Bruni, P., Zecchi Orlandini, S., Francini, F. (2003) Effects of sphingosine 1-phosphate on excitation-contraction coupling in mammalian skeletal muscle. J. Muscle Res. Cell. Motil. 24, 539–554.

64 Danieli-Betto, D., Germinario, E., Esposito, A., Megighian, A., Midrio, M., Ravara, B., Damiani, E., Libera, L. D., Sabbadini, R. A., Betto, R. (2005) Sphingosine 1-phosphate protects mouse extensor digitorum longus skeletal muscle during fatigue. Am. J. Physiol Cell. Physiol. 288, C1367-C1373.

65 Meacci E, Cencetti F, Donati C, Nuti F, Becciolini L, Bruni P. (2004) Sphingosine kinase activity is required for sphingosine-mediated phospholipase D activation in C2C12 myoblasts. Biochem. J. 381, 655–663.

66 Zanin, M., Germinario, E., Dalla Libera, L., Sandonà, D., Sabbadini, R. A., Betto, R., Danieli-Betto, D. (2008) Trophic action of sphingosine 1-phosphate in denervated rat soleus muscle. Am. J. Physiol. Cell. Physiol. 294, C36-C46.

67 Dobrzyń, A., Zendzian-Piotrowska, M., Gorski, J. (2004) Effect of endurance training on the sphingomyelin-signalling pathway activity in the skeletal muscles of the rat. J. Physiol. Pharmacol. 55, 305–313.

68 Ohlson, L. O., Larsson, B., Svärdsudd, K., Welin, L., Eriksson, H., Wilhelmsen, L., Björntorp, P., Tibblin, G. (1985) The influence of body fat distribution on the incidence of diabetes mellitus. 13.5 years of follow-up of the participants in the study of men born in 1913. Diabetes. 34, 1055–1058.

69 McGarry, J. D. (2002) Banting lecture 2001: dysregulation of fatty acid metabolism in the etiology of type 2 diabetes. Diabetes. 51, 7–18.

70 Turinsky, J., O'Sullivan, D. M., Bayly, B. P. (1990) 1,2-Diacylglycerol and ceramide levels in insulin-resistant tissues of the rat in vivo. J. Biol. Chem. 265, 16880–16885.

71 Gorska, M., Dobrzyn, A., Zendzian-Piotrowska, M., Gorski, J. (2004) Effect of streptozotocin-diabetes on the functioning of the sphingomyelin-signalling pathway in skeletal muscles of the rat. Horm. Metab. Res. 36, 14–21.

72 Adams, J. M.2nd, Pratipanawatr, T., Berria, R., Wang, E., DeFronzo, R. A., Sullards, M.C., Mandarino, L. J. (2004) Ceramide content is increased in skeletal muscle from obese insulin-resistant humans. Diabetes. 53, 25–31.

73 Straczkowski M, Kowalska I, Nikolajuk A, Dzienis-Straczkowska S, Kinalska I, Baranowski M, Zendzian-Piotrowska M, Brzezinska Z, Gorski J. (2004) Relationship between insulin sensitivity and sphingomyelin signaling pathway in human skeletal muscle. Diabetes. 53, 1215–1221.

74 Straczkowski, M., Kowalska, I., Baranowski, M., Nikolajuk, A., Otziomek, E., Zabielski, P., Adamska, A., Blachnio, A., Gorski, J., Gorska, M. (2007) Increased skeletal muscle ceramide level in men at risk of developing type 2 diabetes. Diabetologia. 50, 2366–2373.

75 Hajduch, E., Balendran, A., Batty, I. H., Litherland, G. J., Blair, A. S., Downes, C. P., Hundal, H. S. (2001) Ceramide impairs the insulin-dependent membrane recruitment of protein kinase B leading to a loss in downstream signalling in L6 skeletal muscle cells. Diabetologia. 44, 173–183.

76 Zhou, H., Summers, S. A., Birnbaum, M. J., Pittman, R. N. (1998) Inhibition of Akt kinase by cell-permeable ceramide and its implications for ceramide-induced apoptosis. J. Biol. Chem. 273, 16568–16575.

77 Dobrzyń, A., Górski, J. (2002) Effect of acute exercise on the content of free sphinganine and sphingosine in different

skeletal muscle types of the rat. Horm. Metab. Res. 34, 523–529.
78 Helge, J. W., Dobrzyn, A., Saltin, B., Gorski, J. (2004) Exercise and training effects on ceramide metabolism in human skeletal muscle. Exp. Physiol. 89, 119–127.
79 Planavila, A., Alegret, M., Sánchez, R. M., Rodríguez-Calvo, R., Laguna, J. C., Vázquez-Carrera, M. (2005) Increased Akt protein expression is associated with decreased ceramide content in skeletal muscle of troglitazone-treated mice. Biochem. Pharmacol. 69, 1195–1204.
80 Summers, S. A.(2006) Ceramides in insulin resistance and lipotoxicity. Prog. Lipid Res. 45, 42–72.
81 Dobrowsky, R. T., Kamibayashi, C., Mumby, M. C., Hannun, Y. A. (1993) Ceramide activates heterotrimeric protein phosphatase 2A. J. Biol. Chem. 268, 15523–15530.
82 Stratford, S., Hoehn, K. L., Liu, F., Summers, S. A. (2004) Regulation of insulin action by ceramide: dual mechanisms linking ceramide accumulation to the inhibition of Akt/protein kinase B. J. Biol. Chem. 279, 36608–36615.
83 Schmitz-Peiffer, C., Craig, D. L., Biden, T. J. (1999) Ceramide generation is sufficient to account for the inhibition of the insulin-stimulated PKB pathway in C2C12 skeletal muscle cells pretreated with palmitate. J. Biol. Chem. 274, 24202–24210.
84 Chavez, J. A., Summers, S. A. (2003) Characterizing the effects of saturated fatty acids on insulin signaling and ceramide and diacylglycerol accumulation in 3T3-L1 adipocytes and C2C12 myotubes. Arch. Biochem. Biophys. 419, 101–109.
85 Pickersgill, L., Litherland, G. J., Greenberg, A. S., Walker, M., Yeaman, S.J. (2007) Key role for ceramides in mediating insulin resistance in human muscle cells. J. Biol. Chem. 282, 12583–12589.
86 Holland, W. L., Brozinick, J. T., Wang, L. P., Hawkins, E. D., Sargent, K. M., Liu, Y., Narra, K., Hoehn, K. L., Knotts, T. A., Siesky, A., Nelson, D. H., Karathanasis, S. K., Fontenot, G. K., Birnbaum, M. J., Summers, S. A. (2007) Inhibition of ceramide synthesis ameliorates glucocorticoid-, saturated-fat-, and obesity-induced insulin resistance. Cell. Metab. 5, 167–179.
87 Turpin, S. M., Lancaster, G. I., Darby, I., Febbraio, M. A., Watt, M. J. (2006) Apoptosis in skeletal muscle myotubes is induced by ceramides and is positively related to insulin resistance. Am. J. Physiol. Endocrinol. Metab. 291, E1341-E1350.
88 Ma, M. M., Chen, J. L., Wang, G. G., Wang, H., Lu, Y., Li, J. F., Yi, J., Yuan, Y. J., Zhang, Q. W., Mi, J., Wang, L.Sh., Duan, H. F., Wu, C. T. (2007) Sphingosine kinase 1 participates in insulin signalling and regulates glucose metabolism and homeostasis in KK/Ay diabetic mice. Diabetologia. 50, 891–900.
89 Taha, T. A., Kitatani, K., El-Alwani, M., Bielawski, J., Hannun, Y. A., Obeid, L. M. (2006) Loss of sphingosine kinase-1 activates the intrinsic pathway of programmed cell death: modulation of sphingolipid levels and the induction of apoptosis. FASEB J. 20, 482–484.
90 Yamashita, T., Hashiramoto, A., Haluzik, M., Mizukami, H., Beck, S., Norton, A., Kono, M., Tsuji, S., Daniotti, J. L., Werth, N., Sandhoff, R., Sandhoff, K., Proia, R.L. (2003) Enhanced insulin sensitivity in mice lacking ganglioside GM3. Proc. Natl. Acad. Sci. U S A. 100, 3445–3449.
91 Zhao, H., Przybylska, M., Wu, I. H., Zhang, J., Siegel, C., Komarnitsky, S., Yew, N. S., Cheng, S. H. (2007) Inhibiting glycosphingolipid synthesis improves glycemic control and insulin sensitivity in animal models of type 2 diabetes. Diabetes. 56, 1210–1218.
92 Safdar A, Yardley N. J., Snow R, Melov S, Tarnopolsky M. A. (2008) Global and targeted gene expression and protein content in skeletal muscle of young men following short-term creatine monohydrate supplementation. Physiol Genomics. 32, 219–228.
93 Vandenberghe, K., Goris, M., Van Hecke, P., Van Leemputte, M., Vangerven, L., Hespel, P. (1997) Long-term creatine intake is beneficial to muscle performance during resistance training. J. Appl. Physiol. 83, 2055–2263.
94 Volek, J. S., Duncan, N. D., Mazzetti, S. A., Staron, R. S., Putukian, M., Gómez, A.L., Pearson, D. R., Fink, W. J., Kraemer, W. J. (1999) Performance and muscle fiber adaptations to creatine supplementation and heavy resistance training. Med. Sci. Sports. Exerc. 31, 1147–1156.