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Sphingosine Kinase 1 Is Regulated by Peroxisome Proliferator-activated Receptor α in Response to Free Fatty Acids and Is Essential for Skeletal Muscle Interleukin-6 Production and Signaling in Diet-induced Obesity*

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Background: The fatty acid palmitate induces sphingosine kinase 1 (*Sphk1*) activity in skeletal muscle by an unknown mechanism.
Results: SphK1 is transcriptionally up-regulated by peroxisome proliferator-activated receptor α in lipid overload and promotes interleukin (*IL*)-6 expression and signaling.
Conclusion: Palmitate-induced IL-6 is SphK1-dependent and mediates paracrine/autocrine IL-6 signaling in muscle.
Significance: This study identifies a novel role for SphK1 in the context of obesity.

We previously demonstrated that sphingosine kinase 1 (*Sphk1*) expression and activity are up-regulated by exogenous palmitate (PAL) in a skeletal muscle model system and in diet-induced obesity in mice; however, potential functions and *in vivo* relevance of this have not been addressed. Here, we aimed to determine the mechanism by which PAL regulates SphK1 in muscle, and to determine potential roles for its product, sphingosine-1-phosphate (S1P), in muscle biology in the context of obesity. Cloning and analysis of the mouse *Sphk1* promoter revealed a peroxisome proliferator-activated receptor (PPAR) α cis-element that mediated activation of a reporter under control of the *Sphk1* promoter; direct interaction of PPARα was demonstrated by chromatin immunoprecipitation. PAL treatment induced the proinflammatory cytokine interleukin (IL)-6 in a manner dependent on SphK1, and this was attenuated by inhibition of the sphingosine-1-phosphate receptor 3 (S1PR3). Diet-induced obesity in mice demonstrated that *IL-6* expression in muscle, but not adipose tissue, increased in obesity, but this was attenuated in *Sphk1*$^{-/-}$ mice. Moreover, plasma IL-6 levels were significantly decreased in obese *Sphk1*$^{-/-}$ mice relative to obese wild type mice, and muscle, but not adipose tissue IL-6 signaling was activated. These data indicate that PPARα regulates *Sphk1* expression in the context of fatty acid oversupply and links PAL to muscle IL-6 production. Moreover, this function of SphK1 in diet-induced obesity suggests a potential role for SphK1 in obesity-associated pathological outcomes.

The past decades have witnessed an increase in the incidence of obesity and a subsequent increase in associated consequences (1–6). Although the mechanisms linking obesity to downstream pathology remain unclear, elevation in plasma lipids induced by obesity likely plays a role by overloading tissues with precursors for bioactive lipid synthesis including diacylglycerols and ceramides, thus perturbing cell homeostasis (7–9). This general process has been termed "lipotoxicity" (7). In obese individuals, plasma free fatty acids (FFA)[2] can reach levels double those of lean individuals (10). Moreover, elevation of plasma triglycerides also leads to fatty acid oversupply in tissues that express endothelial lipoprotein lipase, including skeletal muscle (11). Skeletal muscle is particularly relevant in this context, as it accounts for ~40% of total body mass and contributes significantly to whole body fatty acid uptake and oxidation (7, 12, 13).

PAL, the most abundant FFA in circulation, has been linked to lipotoxicity in skeletal muscle (4, 14, 15). PAL serves as a substrate for *de novo* sphingolipid synthesis and treatment with exogenous PAL induced *de novo* synthesis in skeletal muscle (6, 16–18). In addition to providing excess substrate for *de novo* synthesis, PAL has been demonstrated to drive sphingolipid synthesis through other mechanisms. These mechanisms

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[2] The abbreviations used are: FFA, plasma free fatty acid; SphK1, sphingosine kinase 1; IL-6, interleukin-6; PAL, palmitate; S1P, sphingosine-1-phosphate; S1PR, S1P receptor; TLR4, Toll-like receptor 4; SOCS3, suppressor of cytokine signaling 3; HFD, high-fat diet; STAT3, signal transducer and activator of transcription 3; FABP, fatty acid-binding protein; qPCR, quantitative PCR.

include the activation of Toll-like receptor 4 (TLR4) (19) and regulation of the enzymes of sphingolipid metabolism. Specifically, we previously demonstrated aberrant regulation of dihydroceramide desaturase and SphK1 in fatty acid-treated cells and/or a mouse model of obesity (6, 20). We hypothesized that these events may play roles in pathophysiological outcomes of obesity. Supporting this notion, sphingolipids are implicated in obesity-induced insulin resistance, disruption of mitochondrial function, and other lipotoxic programs (7–9).

Two isoforms of sphingosine kinase, SphK1 and -2, catalyze phosphorylation of sphingosine to generate S1P. In addition to recently described intracellular targets of S1P (21), S1P signals through G protein-coupled receptors that mediate processes such as chemotaxis, cellular differentiation, proliferation, survival, and inflammation (6, 22–24). SphK1 activity is regulated by several factors including vascular endothelial growth factor (25), epidermal growth factor (26), cytokines, such as tumor necrosis factor α (27), and its metabolite S1P (27, 28); however, little is known regarding its transcriptional regulation. A previous study demonstrated its regulation in hypoxia in U87MG cells by HIF2α (29). However, mechanisms of *Sphk1* transcriptional control in response to other stimuli remain undetermined.

Peroxisome proliferator-activated receptor (PPAR) α is a key transcription factor involved in regulating lipid catabolism including cellular fatty acid uptake, intracellular fatty acid transport, mitochondrial and peroxisomal fatty acid oxidation, and gluconeogenesis (30–32). PPARs are ligand-activated; whereas some controversy exists as to the specific endogenous ligands, studies have demonstrated activation of PPARα-dependent transcription by fatty acids (32–34). Interleukin-6 (IL-6), a cytokine that acts in both pro- and anti-inflammatory pathways (35, 36), is produced by cells of the immune system; however, recent studies have demonstrated that *Il-6* expression and production increase in contracting skeletal muscle (37–39). IL-6 produced by skeletal muscle is the prototype for an emerging class of cytokines known as myokines, which may mediate cross-talk between muscle and other tissues (40, 41). IL-6 is also increased in plasma of obese individuals (42), and correlates with increased levels of plasma FFA (42). These findings suggest IL-6 may play a role in obesity-induced inflammation (41). IL-6 is known to be regulated by fatty acids, and has also been shown to be increased by S1P-induced signaling pathways, although not in the context of metabolic disease (43–45).

Importantly, whereas S1P was demonstrated to be elevated in mouse models of obesity (46, 47), a mechanistic role for SphK1/S1P in obesity remains unknown. Because PAL induced SphK1 in a skeletal muscle model, we hypothesized that SphK1 may regulate IL-6 in obesity. Here we demonstrate that transcriptional regulation of *Sphk1* by PAL occurs through PPARα, placing SphK1 in the context of global lipid metabolism. Moreover, we demonstrate that in diet-induced obesity in mice, SphK1 is required for *Il-6* muscle message, increased plasma IL-6, and muscle-specific activation of IL-6 signaling. These findings suggest a novel mechanistic role for SphK1 in the pathophysiological consequences of obesity.

EXPERIMENTAL PROCEDURES

Materials—Fetal bovine serum, horse serum, 0.5% trypsin EDTA, and Lipofectamine 2000 were from Invitrogen. DMEM with 4 mM L-glutamine was from ATCC (Manassas, VA); GW6471 and WY14,643 were from Sigma; palmitic acid salt (C16:0) was from Matreya, LLC (Pleasant Gap, PA); anti-PPARα antibody was from Abcam (Cambridge, MA); S1P was from Enzo Life Sciences (Plymouth Meeting, PA); JTE013 was from Cayman Chemical (Ann Arbor, MI); and VPC23019 was from Avanti Polar Lipids (Alabaster, AL).

Cell Culture—Mouse C2C12 myoblasts (ATCC) were maintained at 37 °C and 5% CO_2 in DMEM containing 10% FBS. Primary myoblasts were isolated from mice as previously described in Ref. 48. In brief, muscles from the hind limbs of 8-week old C57Bl/6J (WT; The Jackson Laboratory, Bar Harbor, ME) and *Sphk1*$^{-/-}$ mice initially generated by Dr. Richard Proia (49) were maintained in house at the Veterans Affairs animal care facility on a 12-h light cycle. Tissues were dissociated in collagenase/dispase/$CaCl_2$ solution (1.5 units/ml of collagenase D from Fisher Scientific, 2.4 units/ml of dispase II from Invitrogen, and 2.5 mM $CaCl_2$) and incubated at 37 °C for 1 h. Cells were filtered and resuspended in F-10-based primary myoblast growth medium (F-10 nutrient mixture (Invitrogen)) supplemented with 20% FBS, 25 μg/ml of human basic fibroblast growth factor (Invitrogen), and 1% penicillin/streptomycin (Invitrogen). Filtered cells were plated on calf collagen (Fisher Scientific)-coated plates and maintained in F-10-based primary myoblast growth medium for 7 days. Cells were then maintained in F-10/DMEM-based primary myoblast growth medium (40% F-10-based primary myoblast growth medium and 40% DMEM) for an additional 4 days. Confluent C2C12 and primary cells were both differentiated in DMEM supplemented with 10% horse serum for 7 days forming myotubes for utilization in experiments.

FFA Treatment—FFAs were prepared as previously described in Ref. 50. Briefly, palmitate salt (Matreya, LLC) dissolved in 100% ethanol to a concentration of 125 mM was added to serum-free DMEM containing 2% fatty acid-free BSA and 1% FBS to a concentration of 0.75 mM, briefly sonicated, incubated at 55 °C, and cooled to 37 °C. Myoblasts were treated for 8 h and primary myotubes were treated for 16 h.

Cloning of the Sphk1 Promoter—The mouse *Sphk1* promoter was cloned based on sequence homology with the rat *Sphk1* promoter (51, 52). Primers from Integrated DNA Technologies (Coralville, IA) beginning at the −2239 position upstream (5′-GAGGAGTCTCGAGGTTCTGTGTAACCGGA-3′) and the +1592 position downstream (5′-ATCGCTACCATGGTTCAGCTTATCGGT-3′) of the transcription initiation site were used to clone the promoter into the PGL3-basic vector (Promega) utilizing the XhoI and NcoI restriction sites.

Generation of Sphk1 Promoter Deletions—The *Sphk1* promoter was used as a PCR template to generate systematic deletion constructs. The forward primers −1869 (5′-CTCGAGTGTTTATCTCCACCGAAGCGCATAC-3′), −1691 (5′-CTCGAGCGATCATCCGCGGCAGGCAGCATCT-3′), −1333 (5′-CTCGAGTTCGAGGTTCAGTAAGCGCAGACCC-3′), −920 (5′-CTCGAGCCTTGGAGTCGGTGTCAGCCCAGG-

3′), −519 (5′-CTCGAGTACGCGCCTCTCAATGCCAGTT-CTG-3′), and −299 (5′-CTCGAGCAGGGGCTCTGGTT-GGGCACTTTGT-3′) were used with the reverse primer, 5′-ATCGCTACCATGGTTCAGCTTCTTATCCGT-3′, from Integrated DNA Technologies (Coralville, IA). PCR products were cloned into pCR-2.1 TOPO vectors (Invitrogen) and subcloned into PGL3-basic vectors (Promega).

SphK1 Promoter Transfection and Luciferase Assay—The full-length *Sphk1* promoter or the deletion constructs were transfected into C2C12 myoblasts using Lipofectamine 2000 (Invitrogen), according to the manufacturer's protocol. Cells were co-transfected with a LacZ-encoding plasmid as a control for transfection efficiency. Eighteen hours post-transfection, cells were treated with FFA for 8 h and luciferase activity was quantified utilizing the Luciferase Assay Kit (Agilent, Santa Clara, CA), following the manufacturer's instructions. Luciferase data were normalized to β-galactosidase activity as determined by the High Sensitivity β-Galactosidase Kit (Agilent).

Chromatin Immunoprecipitation Assay—C2C12 myoblasts were treated with FFA for 16 h and cross-linking was performed by treatment with 1% formaldehyde solution for 15 min at room temperature. Cross-linking was terminated by the addition of glycine (125 mM). Cells were lysed and chromatin was sheared in a sonicating water bath at ambient temperature, using a 6-s on and 10-s off protocol for 10 cycles. Chromatin was precleared with protein A-agarose/salmon sperm DNA beads (Millipore, Billerica, MA) at 4 °C for 2 h. Precleared chromatin samples were immunoprecipitated with mouse monoclonal anti-PPARα antibody (Abcam, Cambridge, MA) overnight at 4 °C. Immunoprecipitated chromatin was eluted and reverse cross-linked, purified, and subjected to real-time PCR. DNA removed from samples prior to preclearing served as input controls. Real-time primers were designed to amplify the region containing the PPARα responsive element located at the 5′ terminus of the *Sphk1* promoter: sense, 5′-AGCTTCCTTGGGA-GTTTGGTGTCT-3′ and antisense, 5′-TTTCATGGCAAGT-GACCTGAGGGT-3′. Fatty acid-binding protein (FABP) was used as a comparison for PPARα as it is a known PPARα target using primers from SA Biosciences (Valencia, CA). Conventional PCR to amplify the region containing the PPARα responsive element was performed according to standard protocols using the same primers as those used for real-time PCR.

siRNA Transfection—Sixty percent confluent C2C12 myoblasts were transfected with either non-targeting control siRNA or specific siRNAs for *PpaRα*, *S1pr1*, or *S1pr3* siRNAs using Lipofectamine 2000 (Invitrogen) following the manufacturer's protocol. Seventy-two hours post-transfection, cells were treated with BSA or 0.75 mM PAL for 16 h. Total RNA was isolated using the TRIzol reagent (Invitrogen) and utilized for quantitative PCR (qPCR).

Quantitative Real-time PCR—Total RNA from C2C12 myoblasts or primary myotubes was isolated using the TRIzol reagent (Invitrogen) according to the manufacturer's instructions. First-strand cDNA was synthesized from 4 μg of total RNA using the Superscript™ First-strand Synthesis System (Invitrogen). Quantitative real-time PCR was performed on an iCycler System as described in Ref. 6. Mouse *Sphk1*, *Il-6*, *S1pr1*, *S1pr3*, and suppressor of cytokine signaling 3 (*Socs3*) primers were from Integrated DNA Technologies (Coralville, IA). *Gapdh* was used as a reference gene.

Lipidomic Measurement by LC/MS—Lipidomic profiling was performed as previously described (53).

Diet-induced Obesity in Mice—Eight-week old male wild type (Jackson Laboratory, Bar Harbor, ME) and *Sphk1*$^{-/-}$ mice were maintained on control (10% calories from fat) and high-fat (HFD; 60% calories from fat) diets 12 weeks. At the culmination of 12 weeks, mice were sacrificed and skeletal muscle, adipose tissue, and plasma were isolated from the animals for use in future analyses.

Plasma IL-6 Measurements—Changes in plasma IL-6 levels were measured using the Bio-Plex 2200 system (Bio-Rad). Assays were carried out according to the manufacturer's instructions. Briefly, plasma samples from mice on control and a high-fat diet for 12 weeks were diluted 1:4 and incubated in a 96-well plate with magnetic antibody-coupled beads for 30 min at room temperature while shaking. The 96-well plates were washed and incubated with detection antibodies for 30 min, followed by the addition of streptavidin-PE for 10 min. IL-6 levels were measured using the Bio-Plex 2200 system. Each reaction was carried out in duplicate.

Statistics—Statistical significance was determined by Student's *t* test, with a value of $p \leq 0.05$ considered significant.

RESULTS

PAL Induction of S1P Production Occurs through Transcriptional Regulation of SphK1—Our laboratory previously published that PAL increased SphK1 activity and S1P in C2C12 myotubes. In that study, the message for both *Sphk1* and *Sphk2* increased (6). Thus, to clarify which isoform mediated PAL-induced S1P production, we employed primary myoblasts isolated from male wild type (WT) or *Sphk1*$^{-/-}$ mice. Cells were isolated and differentiated to myotubes as described under "Experimental Procedures." Myotubes were treated with 0.75 mM PAL for 16 h. S1P content was near the lower limit of detection, and thus, samples were pooled to determine S1P content by LC/MS. As demonstrated in Fig. 1*A*, S1P production was attenuated in pooled *Sphk1*$^{-/-}$ myotubes basally and in response to PAL treatment ($p = 0.09$), which suggested that S1P production by skeletal muscle derives largely from SphK1. Moreover, consistent with data in the C2C12 cell line, PAL treatment of primary myotubes increased *Sphk1* expression ~2-fold (Fig. 1*B*). Together, these data support that PAL induction of *Sphk1* constitutes the primary route of S1P production in response to PAL treatment, and that this occurs in both immortalized and primary cells.

Studies have indicated that PAL perturbs cell sphingolipid profiles through multiple mechanisms including direct substrate supply (6, 20). A previous report implicated TLR4 in the regulation of the synthesis of ceramide *in vivo* (19); however, the regulation of S1P generation was not addressed. Because ceramides serve as metabolic precursors for S1P synthesis, we hypothesized that PAL may increase S1P through TLR4-mediated ceramide increase. To test this, we transfected C2C12 cells with siRNA targeted to *Tlr4* or negative control siRNA. Knockdown of *Tlr4* did not attenuate S1P production in response to PAL treatment (data not shown). Moreover, ceramides, previ-

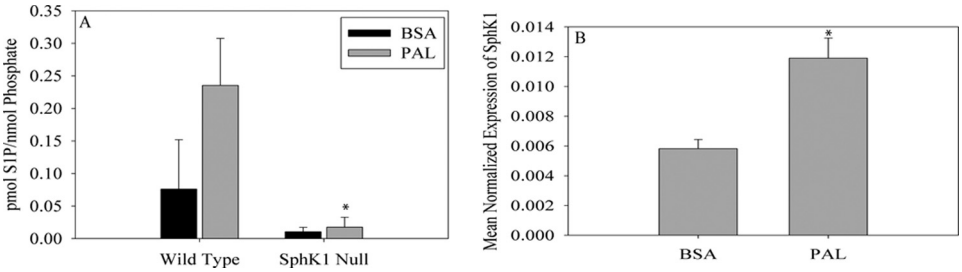

FIGURE 1. **PAL-induced S1P production is SphK1 dependent.** Myoblasts were isolated from C57Bl/6J and *Sphk1*$^{-/-}$ mice and differentiated to myotubes. Following a 16-h treatment with BSA or 0.75 mM PAL, cells were harvested and analyzed. *A*, three sets of isolated myotubes were pooled for the measurement of total S1P. S1P content was determined by LC/MS. Data are represented as mean ± S.E. ($n = 2$). *, $p = 0.09$ *versus* PAL WT. *B*, *Sphk1* mRNA expression was determined in wild type myotubes by qPCR. Data are expressed as mean ± S.E. ($n = 3$). *, $p < 0.05$ *versus* BSA-treated cells.

ously suggested as downstream of TLR4 signaling in an *in vivo* model (19), were not attenuated in *Tlr4* knockdown cells (data not shown), suggesting that at least some PAL-mediated regulation of sphingolipid metabolism in skeletal muscle may occur independently of TLR4 receptor signaling.

PAL Induces SphK1 Promoter Activity—Our previous studies indicated that the *Sphk1* message increased upon treatment with PAL, which could be due to transcriptional regulation and/or mRNA stability. To distinguish between these possibilities, C2C12 myoblasts were treated with 0.75 mM PAL in the presence and absence of 1 μM actinomycin D for 16 h. Data demonstrated that the PAL-induced *Sphk1* message increase was significantly attenuated in the presence of actinomycin D (Fig. 2*A*), indicating a requirement for transcription. Thus, we hypothesized that PAL caused *Sphk1* promoter activation. To test this, the mouse *Sphk1* promoter was cloned based on sequence homology with the published rat promoter sequence as described under "Experimental Procedures" (51, 52). The two sequences were compared using the BLAST program, using the 5-kb regions flanking the 5′ end of the SphK1 coding sequences. Although the overall sequences shared low homology, a number of key regulatory regions in the rat promoter were conserved in the mouse promoter sequence (Fig. 2*B*). For example, a 55-bp region of the rat promoter, which was shown to be necessary and sufficient for NGF-induced *Sphk1* exon D expression, also appeared at −387 to −256 in the mouse sequence. Moreover, region III, an upstream region from the −3283 to −2991 position in the rat sequence, and which was demonstrated to be particularly sensitive to methylation, was also identified at positions −1543 to the transcription initiation site in the murine promoter sequence. Thus, these regions were used for orientation in cloning the mouse promoter through PCR amplification of wild type genomic DNA using the primers indicated under "Experimental Procedures." Amplification resulted in a 2064-bp fragment that was cloned into TOPO-TA 2.1 vector and then subcloned into the PGL3-Basic luciferase reporter vector.

C2C12 myoblasts were transfected with the PGL3-*Sphk1* luciferase vector, treated for 8 h with 0.75 mM PAL, and luciferase activity was measured. As demonstrated in Fig. 2*C*, *Sphk1* promoter activation occurred in these cells under basal conditions, reflected by a 4-fold increase in luciferase activity, which suggests that these cells basally transcribe *Sphk1*. Moreover, PAL treatment increased *Sphk1* promoter activity 8.5-fold relative to BSA-treated PGL3-*Sphk1*-transfected cells, which further supported the hypothesis that PAL increases *Sphk1* message and activity through increasing its transcription.

To identify potential regions of the *Sphk1* promoter that mediated PAL-induced transcription, systematic promoter deletion constructs were generated in the PGL3 basic luciferase reporter vector; these constructs were used to transfect C2C12 skeletal muscle myoblasts (Fig. 2*D*). Transfected cells were treated with PAL or BSA as above and luciferase activity was quantified. Data indicated that deletion of −2068 to −1869 of the promoter prevented PAL-induced promoter activity (Fig. 2*E*), thus suggesting that this region may contain a PAL-responsive regulatory element. To identify potential cis-acting regulatory elements, the region was analyzed using the ALGGEN-PROMO transcription factor regulatory element prediction software (54), which yielded 3 potential transcription factor-binding domains in the region between the −2068 and −1869 positions (Fig. 2*B*). The potential cis-acting regulatory elements identified were c-Jun and c-Fos, which make up the AP-1 transcription factor, and a PPARα responsive element. To evaluate the contribution of these transcription factors to *Sphk1* gene transcription, siRNA was used to knockdown c-*Fos*, c-*Jun*, and *Pparα*. Interestingly, knockdown of the *Ap-1* complex diminished basal *Sphk1* expression, perhaps suggesting a role for *Ap-1* in basal transcription of *Sphk1*, but had no effect on PAL-induced *Sphk1* expression (data not shown). In contrast, transfection with *Pparα* siRNA resulted in a 75% reduction in *Pparα* message (Fig. 3*A*), which was sufficient to result in a significant decrease in PAL-induced *Sphk1* expression (Fig. 3*B*).

To further test whether PPARα mediated the induction of *Sphk1* by PAL, we treated myoblasts transfected with the *Sphk1* promoter construct with PAL in the presence of GW6471, a potent PPARα antagonist. Data showed that GW6471 attenuated PAL-induced *Sphk1* promoter activity at very low doses (Fig. 3*C*), further supporting that PPARα mediates PAL stimulation of *Sphk1* transcription. To test whether this mechanism may also apply to activation of the endogenous *Sphk1* promoter, we tested inhibition of the PAL-induced increase in a message encoding *Sphk1* in isolated primary myotubes (Fig. 3*D*). These data showed a similar pattern to the luciferase data, suggesting that PPARα-dependent mechanisms were similar whether assessed by reporter gene strategies or direct measurement of endogenous *Sphk1* message.

Although these data support that PAL stimulated PPARα-dependent activation of *Sphk1* transcription, it was still unclear

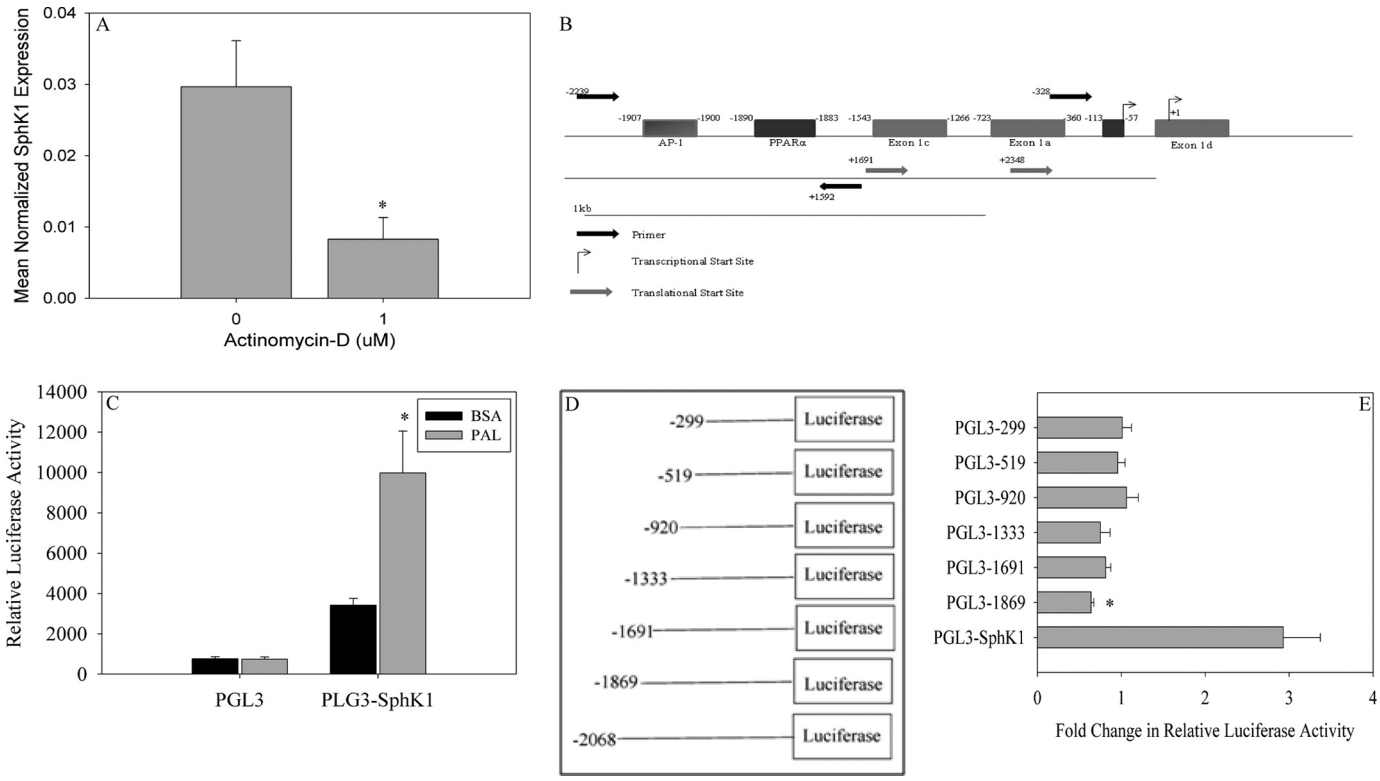

FIGURE 2. **PAL induces SphK1 through activation of the promoter.** *A*, to evaluate the role of transcription in PAL-induced *Sphk1* expression, C2C12 myoblasts were treated with 0.75 mM PAL in the presence or absence of 1 μM actinomycin D for 16 h. Following treatment, cells were harvested and the expression of *Sphk1* was analyzed by qPCR. Data are presented as mean normalized expression ± S.E. ($n = 3$). *, $p < 0.01$ *versus* non-actinomycin D-treated cells. *B*, the murine *Sphk1* promoter was cloned based on sequence homology with the rat *Sphk1* promoter. The cloned promoter was cloned into a PGL3 basic vector for further use. The region of the promoter necessary for PAL-induced *Sphk1* promoter activation was analyzed for transcription factor responsive elements using the transcription factor prediction model ALGGEN-PROMO (54, 56). *C*, C2C12 myoblasts were transfected with empty PGL3 or the PGL3-*Sphk1* vector. Cells were treated with BSA or 0.75 mM PAL 18 h post-transfection for 8 h. Cells were assayed for relative luciferase activity and normalized to β-galactosidase activity. Data are presented as fold-change in relative normalized luciferase activity ± S.E. ($n = 3$). *, $p < 0.05$ *versus* PGL3-*Sphk1* BSA cells. *D*, systematic deletions were generated of the *Sphk1* promoter and cloned into the PGL3 vector. *E*, previously generated deletion constructs were transfected into C2C12 myoblasts. Eighteen hours post-transfection, cells were treated with BSA or 0.75 mM PAL for 8 h and assayed for relative luciferase activity. Measured luciferase activity was normalized to β-galactosidase activity. Data are presented as fold-change in normalized relative luciferase activity ± *S.E.*, ($n = 6$). *, $p < 0.05$ *versus* PGL3-*Sphk1*.

whether this occurred as a result of direct interaction of PPARα with the *Sphk1* promoter, or through downstream PPARα-dependent events. Thus, we tested interaction of PPARα with the *Sphk1* promoter *in situ* using a chromatin immunoprecipitation (ChIP) strategy. In brief, C2C12 myoblasts were treated with 0.75 mM PAL or BSA vehicle for 8 h. As described in detail under "Experimental Procedures," cells were cross-linked, DNA was sheared, a 100-μl aliquot was retained for normalization, whereas the remainder of the chromatin was cleared and utilized for immunoprecipitation. DNA was precipitated from the immunoprecipitate and input samples and utilized for real-time PCR. Indeed, PAL induced a significant increase in the association of PPARα with the *Sphk1* promoter (Fig. 3*E*; representative gel of conventional PCR products shown in Fig. 3*F*) relative to BSA alone treated cells. PPARα has been shown to regulate several genes, including those that participate in fatty acid metabolism. One such gene, *Fabp*, was utilized in the ChIP analysis to serve as positive control and as a comparison for PPARα pull down (Fig. 3*E*). Analysis of ChIP DNA demonstrated that PAL also promoted *Fabp* association with PPARα. Together, these data indicate activation of PPARα by PAL and establish the *Sphk1* gene as a transcriptional target of PPARα in the context of PAL treatment.

PAL Induces Interleukin-6 Expression—Although these data establish the involvement of fatty acids in the induction of *Sphk1* in skeletal muscle, potential roles for this event remain unknown. Given the emerging roles of IL-6 in pathophysiological processes associated with obesity (42, 55) as well as the key roles of IL-6 in muscle (37, 38, 57), we sought to test whether PAL treatment would induce muscle *Il-6* through *Sphk1*. Consistent with previous findings (36, 58), treatment with PAL significantly increased *Il-6* mRNA expression in both wild type primary and C2C12 myotubes (Fig. 4, , *A* and *B*). To test whether this occurred through a SphK1-dependent mechanism, we utilized primary myoblasts isolated from *Sphk1*$^{-/-}$ and WT mice as described under "Experimental Procedures." Myotubes were treated with 0.75 mM PAL and *Il-6* mRNA was measured using quantitative real-time PCR. As shown in Fig. 4*C*, PAL induced a significant increase in *Il-6* expression in WT cells. In contrast, although *Sphk1*$^{-/-}$ cells basally expressed *Il-6*, its expression in response to PAL was significantly attenuated, indicating a requirement for *Sphk1* for PAL-induced *Il-6* expression under these conditions. Taken in total, these data suggest a novel role for SphK1/S1P in mediating the induction of *Il-6* by PAL in skeletal muscle.

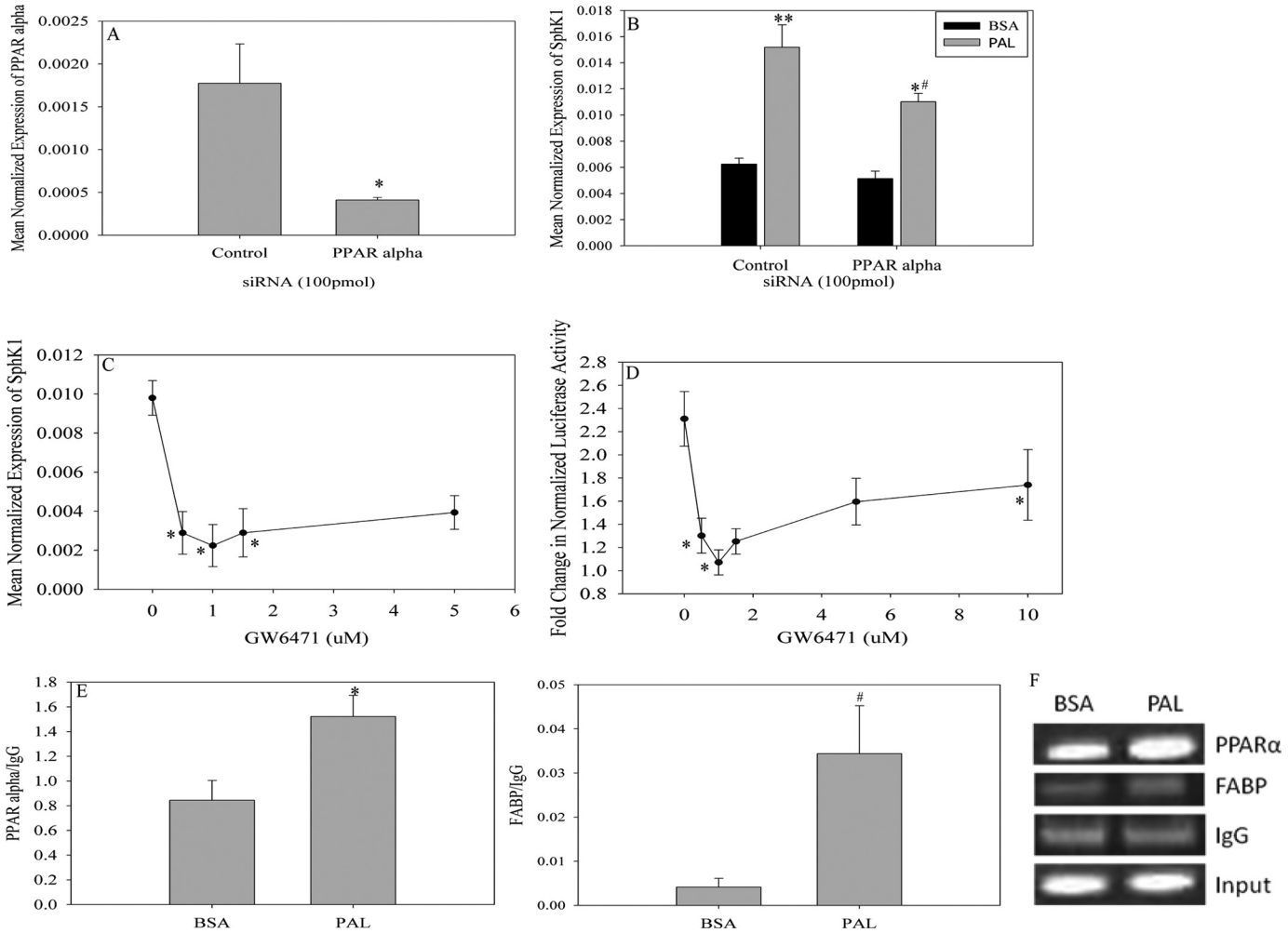

FIGURE 3. **PAL-induced *Sphk1* expression is PPARα dependent.** Analysis of the region indicated by the deletion model to be important in PAL-induced *Sphk1* promoter activity indicated the presence of a *Pparα* responsive element. To assess the importance of this site in PAL-induced *Sphk1* promoter activity. *A*, C2C12 myoblasts were transfected with 100 pmol of control or *Pparα* siRNA. Seventy-two hours post-transfection, cells were harvested and assessed for knockdown efficiency by qPCR. Data are presented as mean normalized expression ± S.E. ($n = 3$). *, $p < 0.01$ *versus* control. *B*, siRNA-transfected cells were treated with BSA or 0.75 mM PAL for 16 h. Cells were harvested and analyzed for *Sphk1* mRNA expression. Data are presented as mean normalized expression ± S.E. ($n = 3$). *, $p < 0.01$ *versus* PAL control; **, $p < 0.01$ *versus* BSA control; #, $p < 0.001$ *versus* BSA *Pparα*. *C*, C2C12 myoblasts were transfected with the *Sphk1* promoter vector and treated 18 h post-transfection with BSA or 0.75 mM PAL for 8 h in the presence of GW6471, a potent PPARα antagonist. Data are expressed as fold-change in normalized relative luciferase activity ± S.E. ($n = 3$); *, $p < 0.05$ *versus* non-GW6471 treated cells. *D*, myoblasts were isolated from WT mice and differentiated into myotubes. Myotubes were treated with 0.75 mM PAL in the presence of GW6471 for 8 h. *Sphk1* expression was determined by qPCR. Data are expressed as mean ± S.E. ($n = 3$). *, $p < 0.05$ *versus* non-GW6471 treated cells. *E*, to establish that PPARα associates with the *Sphk1* promoter, C2C12 myoblasts were treated for 16 h with BSA or 0.75 mM PAL. The cells were utilized for ChIP assays as described under "Experimental Procedures." Data are presented as the ratio of PPARα to background (IgG) ± S.E. ($n = 5$). *, $p < 0.01$ *versus* BSA. The presence of FABP was evaluated for comparison to PPARα pulldown. Data are presented as ratio of FABP to background (IgG) ± S.E. ($n = 5$). #, $p < 0.05$ *versus* BSA. *F*, DNA obtained from ChIP assays were utilized for conventional PCR. Following the PCR, the resulting products were visualized using a 10% native polyacrylamide gel. The gel pictured is a representative of 5 independent experiments.

PAL-induced IL-6 Production Is Dependent on S1P Receptor 3—S1P mediates many processes including tissue inflammation (59). Most identified functions of S1P result from the activation of G protein-coupled S1P receptors of which there are 5 isoforms (S1PR1–S1PR5) (60). Previous studies indicate that skeletal muscle expresses S1PR1, S1PR2, and S1PR3 (61, 62). To determine which receptor may mediate SphK1-dependent IL-6 generation in response to PAL treatment, cells were pre-treated with 1 μM VPC23019, a S1PR1 and S1PR3 antagonist, or 1 μM JTE013, a S1PR2 antagonist, for 1 h. Cells were then treated with 0.5 μM S1P, harvested over time, and the *Il-6* message was measured. As demonstrated in Fig. 5*A*, exogenously added S1P induced *Il-6* expression, further supporting a role for S1P signaling through cell surface receptors in this process. Pretreatment with JTE013 had no effect on S1P-dependent *Il-6* induction (data not shown); however, pre-treatment with VPC23019 blocked S1P induction of IL-6 (Fig. 5*A*). The expression of *S1pr1* and *S1pr3* in response to PAL treatment was evaluated and these data demonstrated that there was no significant difference in expression of either receptor in response to PAL treatment (data not shown).

These data suggested that PAL-induced *Il-6* expression can be attributed to S1P activation of S1PR1 or S1PR3. To distinguish between these possibilities, we employed siRNA directed toward either receptor. Transfection with siRNAs resulted in approximately a 60 and 80% reduction in *S1pr1* and *S1pr3* message, respectively (Fig. 5*B*). Cells were treated with S1P and the *Il-6* message was quantified by qPCR as described above. Data

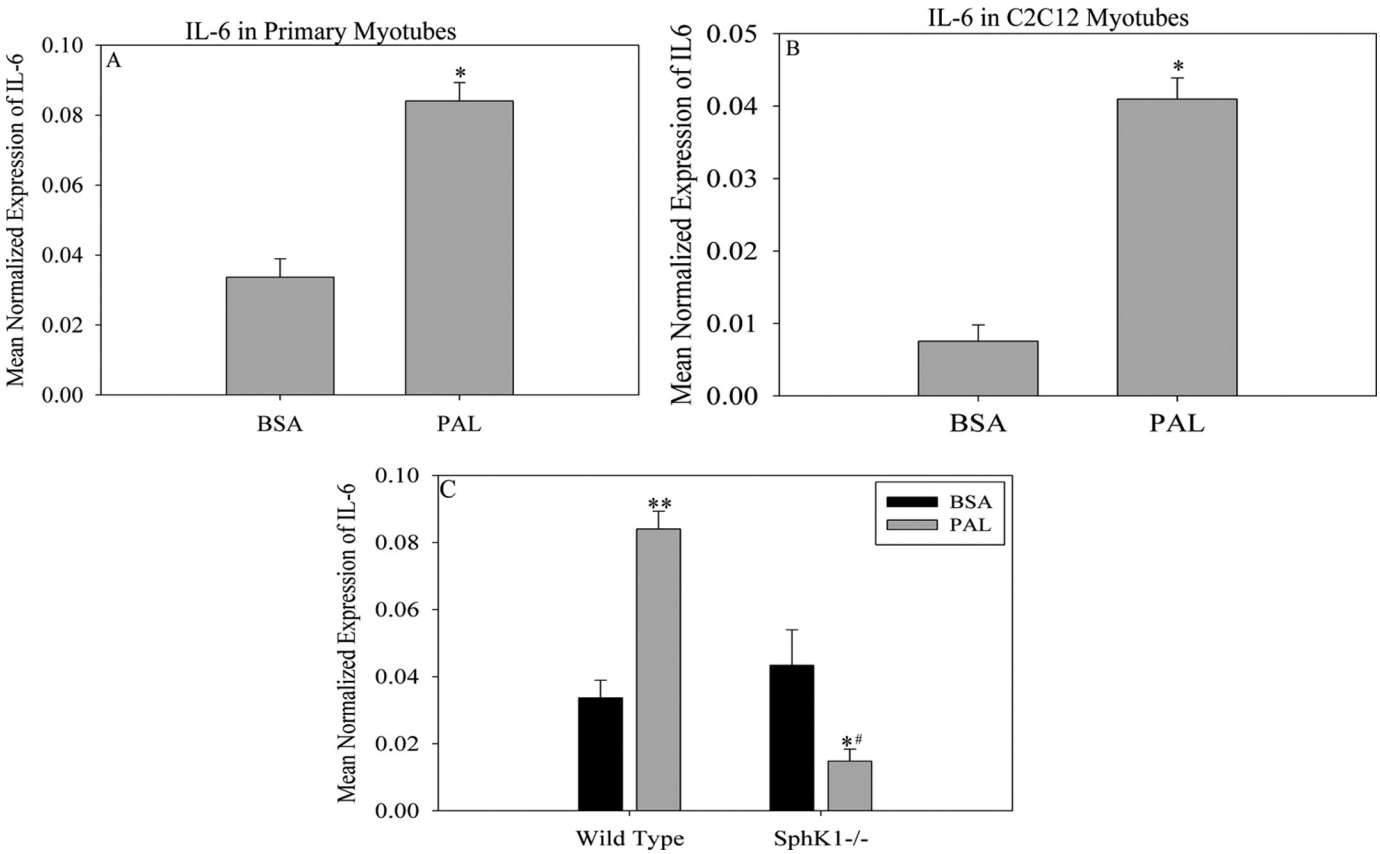

FIGURE 4. **PAL-induced *Il-6* expression is Sphk1 dependent.** The effect of PAL on IL-6, a downstream product of S1P-induced signaling pathways, was assessed in (*A*) primary myotubes and (*B*) C2C12 myotubes. *Il-6* expression was determined by qPCR. Data are presented as mean ± S.E. ($n = 3$). *, $p < 0.01$ *versus* BSA; #, $p < 0.05$ *versus* BSA. *C*, to establish that PAL-induced *Il-6* expression is *Sphk1* dependent, myoblasts were isolated from C57Bl/6J and *Sphk1*$^{-/-}$ mice and differentiated into myotubes. Myotubes were treated with BSA or 0.75 mM PAL for 16 h. IL-6 expression was determined by qPCR. Data are presented as mean ± S.E. ($n = 3$). **, $p < 0.05$ *versus* WT BSA; *, $p < 0.01$ *versus* WT PAL; #, $p < 0.05$ *versus Sphk1*$^{-/-}$ BSA.

demonstrated that knockdown of the *S1pr3* receptor, but not knockdown of the *S1pr1* receptor, attenuated S1P-induced *Il-6* (Fig. 5*C*), suggesting a role for this receptor in *Il-6* induction by PAL.

Sphk1$^{-/-}$ *Mice Are Protected from Obesity-induced Increase in Plasma IL-6*—Fatty acid treatment is used to model lipid oversupply in obesity; however, whereas this may effectively facilitate mechanistic studies, it is limited in its relevance to obesity *in vivo*, which perturbs not only levels of fatty acids but also plasma triacylglycerols, an abundant source of fatty acids in organs such as heart and skeletal muscle, which contain abundant lipoprotein lipase in vascular endothelium (63). Moreover, plasma contains not only PAL, but complex mixtures of fatty acids; both amounts and profiles of these lipids are perturbed in obesity (10). Thus, to test the *in vivo* relevance of the findings in the cell studies above, we placed 8-week-old male WT and *Sphk1*$^{-/-}$ mice on HFD obesogenic or low-fat isocaloric control diets for 12 weeks. *Sphk1*$^{-/-}$ animals maintained on the obesogenic diets gained significantly more weight than their WT counterparts (Fig. 6*A*). To determine the underlying mechanisms for this, food intake was monitored and we found no differences in food intake between WT and *Sphk1*$^{-/-}$ animals. Thus, we analyzed locomotor activity in *Sphk1*$^{-/-}$, which we found was only 25% of that observed in WT mice irrespective of diet (data not shown). Although the underlying mechanism for this remains unknown, it may explain the increased weight gain in the *Sphk1*$^{-/-}$ mice.

To determine whether a high-fat diet induced muscle *Il-6* in a Sphk1-dependent manner *in vivo*, IL-6 mRNA was quantified in skeletal muscle from high-fat diet fed mice of each genotype. Data demonstrated that high-fat feeding induced IL-6 expression in skeletal muscle in WT, but not *Sphk1*$^{-/-}$ animals (Fig. 6*B*).

Adipose tissue and skeletal muscle constitute major tissue sources of plasma IL-6, and thus, to test whether obesity increased adipose IL-6 in a Sphk1-dependent manner, *Il-6* mRNA was quantified in adipose tissue from high-fat diet fed WT and *Sphk1*$^{-/-}$ animals. As shown in Fig. 6*C*, high-fat feeding does not induce *Il-6* expression in adipose tissue as there were no differences between the control and high-fat diet in the *Sphk1*$^{-/-}$ mice, suggesting a muscle-specific role for Sphk1 in *Il-6* production in obesity. To test whether this change in *Il-6* message in skeletal muscle had an impact on circulating IL-6, plasma was prepared and used for Bioplex analysis as described above to measure IL-6 content. These data demonstrated that plasma IL-6 was increased 3-fold in WT mice placed on the obesogenic diet relative to controls, consistent with the magnitude of *Il-6* message increase in cultured skeletal muscle cells. Interestingly, although basal IL-6 was elevated 3.5-fold in *Sphk1*$^{-/-}$ mice, the obesogenic diet produced no further

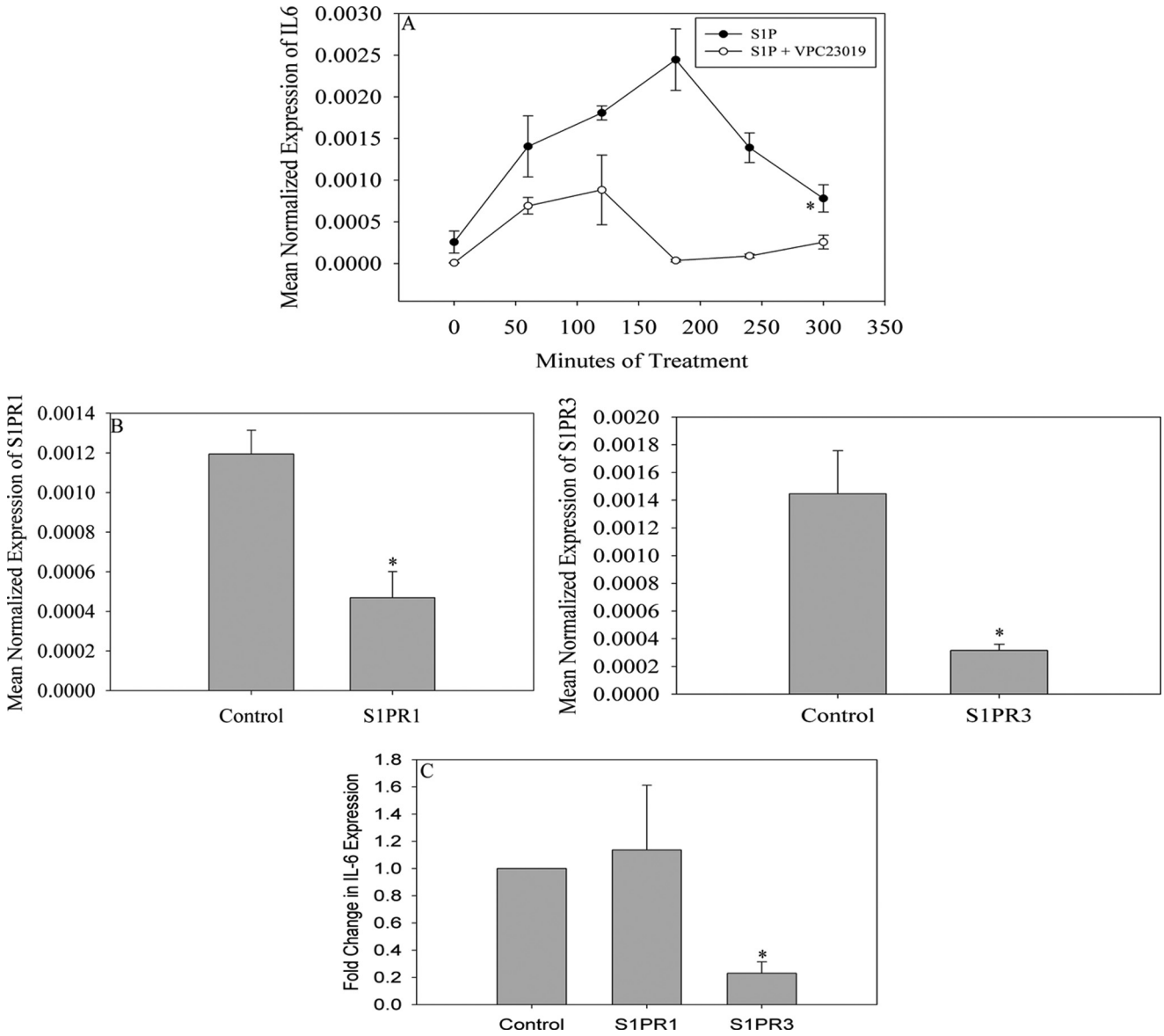

FIGURE 5. **PAL-induced IL-6 expression is S1PR$_3$ dependent.** *A*, to evaluate if the activation of the S1P receptors expressed in skeletal muscle was required for PAL-induced *Il-6* expression, C2C12 myoblasts were treated with S1P alone or pretreated for 1 h with 1 μM VPC23019, a S1P1,3 antagonist, followed by treatment with 0.5 μM S1P for 3 h. Data are presented as mean ± S.E. ($n = 2$). *, $p < 0.05$. *B*, to determine which of the two antagonized receptors is required for S1P-induced *Il-6* expression, C2C12 myoblasts were transfected with 100 pmol of non-targeting control, *S1pr1* or *S1pr3* siRNA. Seventy-two hours post-transfection, cells were serum starved overnight and treated with 0.5 μM S1P for 3 h. The mRNA expression of both target genes was determined by qPCR. Data are expressed as mean normalized expression ± S.E. ($n = 3$). *, $p < 0.01$ *versus* control cells. *C*, upon confirmation of sufficient knockdown of both S1PRs, *Il-6* expression in response to 0.5 μM S1P was evaluated was determined by qPCR. Data are expressed as fold-change in mean normalized expression as compared with the control ± S.E. ($n = 3$). *, $p < 0.01$ *versus* control.

induction (Fig. 6*D*). These data together with the expression data suggest that IL-6 production in muscle in response to high-fat feeding is regulated by Sphk1, and the increase in plasma IL-6 associated with obesity may derive from skeletal muscle, but not adipose.

IL-6 plays roles in systemic inflammation, but a recently proposed hypothesis suggests that IL-6 from skeletal muscle mediates skeletal-adipose cross-talk (41). Specifically, in exercise, muscle releases IL-6, and the purpose of this activity has been speculated to be activation of signaling downstream for the IL-6 receptor in adipose, ultimately stimulating adipocyte lipolysis to temporarily increase plasma FFA, thus restoring energy to depleted tissues. Because obesity leads to chronic elevation of plasma FFA, we hypothesized that SphK1-dependent IL-6 may mediate a similar paradigm in cross-talk between muscle and adipose in this context. Thus, we tested whether adipose IL-6 signaling was activated in the diet-induced obese mice, and if so, whether this required SphK1. Classical signaling of IL-6 occurs through the IL-6 receptor and results in the transcription of *Socs3* in both muscle and adipose (64). Thus, as a readout of IL-6 signaling, the *Socs3* message was evaluated in adipose tissue and skeletal muscle from mice maintained on the control and high-fat diets. In adipose tissue, obesity did not induce *Socs3* expression (Fig. 7*A*), and *Socs3* mRNA levels were similar in mice of both genotypes, suggesting that IL-6 signaling in response to high-fat feeding did not occur in this tissue.

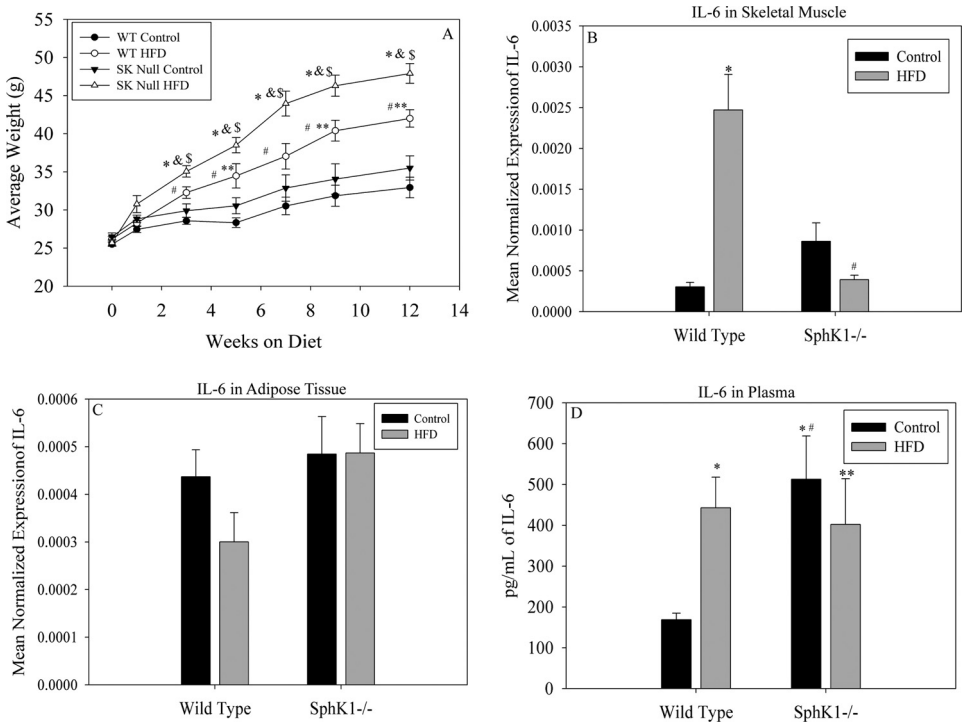

FIGURE 6. **Diet-induced obesity promotes *Il-6* expression in skeletal muscle and is attenuated in *Sphk1*$^{-/-}$ mice.** Eight-week-old male C57Bl/6J and *Sphk1*$^{-/-}$ mice were maintained on obesogenic or low-fat control diets for 12 weeks. *A*, mice maintained on the diets were weighed biweekly. Data are presented as average weight ± S.E., $n \geq 4$ mice per diet group; #, $p < 0.01$ *versus* wild type control; *, $p < 0.01$ *versus Sphk1*$^{-/-}$ HFD; **, $p < 0.05$ *versus Sphk1*$^{-/-}$ control; &, $p < 0.05$ *versus* wild type HFD; $, $p < 0.01$ *versus Sphk1*$^{-/-}$ control. *B*, total skeletal muscle was removed from the hind limbs of diet-fed mice. *Il-6* expression was evaluated using qPCR, *, $p < 0.05$ *versus* WT control; #, $p < 0.01$ *versus* WT HFD. *C*, adipose tissue was removed from the abdomen of mice maintained on the diet and *Il-6* expression was determined by qPCR. All qPCR data in *B* and *C* are presented as mean normalized expression ($n = 4$) ± S.E. *D*, plasma was isolated post-mortem from diet-fed mice and analyzed for IL-6 content via Bioplex 2200 System assay. Data are presented as mean picograms of IL-6/ml of plasma ± S.E., $n \geq 4$ mice per diet group; **, $p < 0.05$ *versus* WT HFD; #, $p < 0.05$ *versus Sphk1*$^{-/-}$ HFD; *, $p < 0.05$ *versus* WT control.

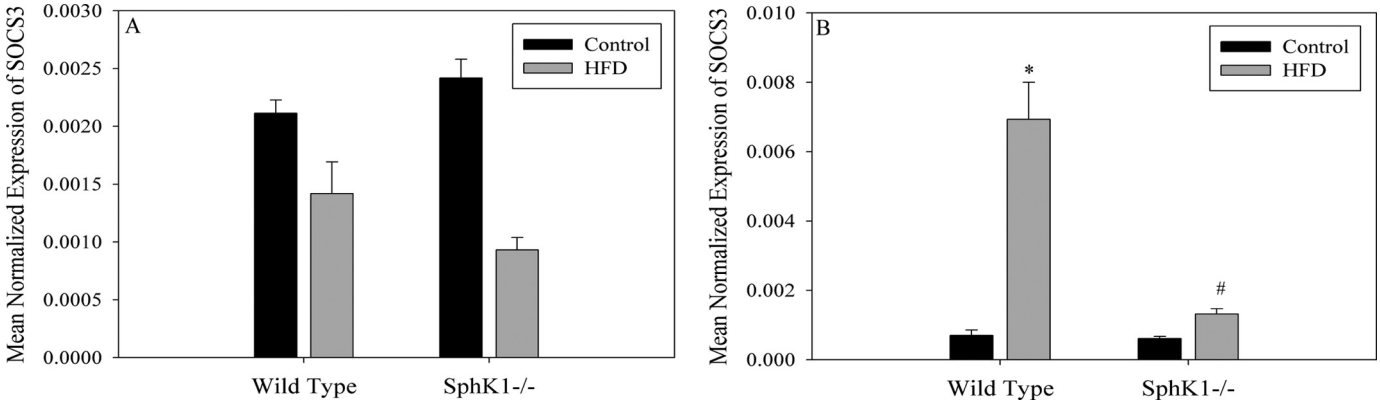

FIGURE 7. **Diet-induced obesity induces the expression of *Socs3* in skeletal muscle.** *A*, adipose tissue was taken from the abdomen of wild type and *Sphk1*$^{-/-}$ mice maintained on the high fat and control diets. Total RNA was extracted and cDNA was synthesized. *Socs3* expression was determined by qPCR. Data are presented as mean normalized expression ± S.E. ($n = 4$). *B*, *Socs3* mRNA expression in skeletal muscle from mice maintained on the diets was determined by qPCR. Data are presented as mean normalized expression ± S.E. ($n = 4$); *, $p < 0.05$ *versus* WT control; #, $p < 0.05$ *versus Sphk1*$^{-/-}$ control.

Analysis of IL-6 signaling targets in skeletal muscle, however, demonstrated that the obesogenic diet induced the expression of *Socs3* in a SphK1-dependent manner (Fig. 7*B*), suggesting that IL-6 signaling was induced in muscle in high-fat feeding, and was attenuated by the absence of SphK1. These data suggest that under these conditions, muscle-derived IL-6 may act in a paracrine fashion by promoting IL-6 signaling in skeletal muscle. Together these data suggest that muscle constitutes a significant source of plasma IL-6 in response to high-fat feeding in mice, and that in this context, muscle IL-6 acts in a paracrine manner and is SphK1- and S1PR3-dependent, thus supporting relevance and tissue specificity of the PAL-SphK1-S1P-S1PR3-IL-6 pathway in obesity.

DISCUSSION

Studies from our laboratory have demonstrated that treatment of mouse skeletal muscle cells with exogenous PAL increased *Sphk1* message (6). PAL-induced SphK1 has also been recently demonstrated to occur similarly in INS-1 pancreatic β cells (65), and thus, this activity of palmitate may be relevant to numerous tissues and organs *in vivo* that succumb to pathology upon fatty acid oversupply.

Post-transcriptional and post-translational regulation of SphK1 has been well characterized (23); for example, SphK1 activation is mediated by ligands of G-protein-coupled receptors (23, 66), phosphorylation of tyrosine receptors (67), TNFα (27), vitamin D3 (68), FBS (66), and the product of SphK1 activity, S1P (23). Moreover, whereas a few studies have revealed transcriptional regulation of *Sphk1* by cis-elements within the human and rat promoters, specifically, binding sites for HIF-1, AP-2, and Sp1 (52, 69), regulation of SphK1 in the context of obesity has not been addressed. The purpose of this study was to determine underlying mechanisms for fatty acid-mediated induction of SphK1 and potential functions in skeletal muscle in obesity.

The data presented in this article demonstrates that PAL-induced S1P production is Sphk1 dependent and that PAL promotes transcription of *Sphk1* through the activation of its promoter. We have also demonstrated that PPARα is necessary for PAL-induced *Sphk1* transcription. Additionally, the data demonstrated that PAL induces expression of *Il-6* through S1PR3. Moreover, we demonstrated that a target of IL-6 signaling, *Socs3*, is increased in skeletal muscle, but not adipose tissue of diet-induced obese mice, supporting a muscle-specific role for this pathway *in vivo*.

Pparα expression has been found in tissues with high levels of fatty acid catabolism. These tissues include liver, heart, kidney, the small and large intestine, and skeletal muscle (70, 71). There are several known ligands of PPARα; in the context of metabolic syndrome and obesity, fatty acids have been shown to serve as biological, or endogenous, PPARα ligands (70, 72). Polyunsaturated fatty acids have been shown to be potent PPARα activators; however, it has come to light that saturated fatty acids can also induce PPARα activation (73, 74). However, data here do not preclude a metabolite of PAL in PPARα activation, for example, PAL-derived phosphatidylcholine species, which were recently reported as endogenous ligands of PPARα in liver (75), may also increase in muscle in this model. PPARα-dependent transcriptional regulation results in increased lipid oxidation and decreased triacylglycerol accumulation (76, 77). As PPARα is a central regulator of lipid metabolism, these data place SphK1 in the broad context of lipid metabolism.

IL-6 has been shown to act as a proinflammatory cytokine, playing a role in several diseases including inflammatory bowel disease (78, 79), colorectal cancer (79, 80), and type 2 diabetes (42, 55). In recent years, skeletal muscle has been identified as a site for cytokine, including IL-6, production (37), placing IL-6 in a class of cytokines secreted by skeletal muscle and thus termed "myokines" (40, 41). Previous studies have demonstrated that the expression and production of IL-6 is sensitive to elevations of FFA, more specifically PAL, in skeletal muscle (36, 58, 81). PAL has been shown to promote the activation of NFκB to induce IL-6 generation in rat skeletal muscle (83). In C2C12 myotubes, PAL treatment was shown to robustly induce IL-6 as early as 8 h with maximal IL-6 production achieved following 16 h of treatment (84). Consistent with our findings that high-fat feeding induces *Il-6* in the skeletal muscle of WT mice, Reyna *et al.* (85) demonstrated that IL-6 expression was increased in the skeletal muscle of obese individuals. These studies support that data presented here may bear relevance to pathophysiological outcomes of obesity in humans.

Adipose tissue-derived IL-6 accounts for a large proportion of circulating IL-6 (86), with visceral or omental adipose tissue accounting for a larger proportion than that of subcutaneous fat depots (87). Consistent with our data, studies have demonstrated that 12 weeks of high-fat feeding did not induce *Il-6* mRNA expression in adipose tissue (88, 89), but rather occurred at time points beyond 16 weeks (90). Whether this would occur in a manner dependent on SphK1 remains unknown. However, because our data demonstrated that circulating IL-6 was elevated in response to high-fat feeding (Fig. 6*D*), concomitant with *Il-6* message induction of skeletal muscle, another major tissue source of IL-6, we conclude that muscle constitutes a major determinant of plasma IL-6 under our experimental conditions. We show here that a well established target of IL-6 signaling, *Socs3*, is elevated in muscle but not adipose tissue in high-fat feeding (Fig. 7*A*), and thus conclude that IL-6 may signal in an autocrine/paracrine manner in this context. This conclusion gains further support from studies that demonstrate that, whereas adipose tissue accounts for a large proportion of circulating IL-6, IL-6 signaling in adipose tissue is not well established (91).

In a similar vein, *in vivo* induction of skeletal muscle IL-6 by high-fat feeding (Fig. 6) was of a higher magnitude than in palmitate-treated isolated cells or cell lines (Fig. 4). We suspect that some of the additional increase observed in tissue homogenates may derive from other cell types in the homogenates including myoblasts and/or immune cells. However, because muscle and adipose tissue are major tissues determining plasma IL-6 levels, and that *Il-6* message in adipose did not change in a manner dependent on SphK1, muscle IL-6 likely underlies the findings in plasma, which demonstrated an increase in high-fat feeding that was attenuated in *Sphk1*$^{-/-}$ animals. This is a consistent with the observation that changes in message level in muscles of 2–3-fold are similar to the magnitude of the increase we observed in plasma.

IL-6 signaling occurs in part through increased phosphorylation of STAT3, a transcription factor that regulates the expression of IL-6 target genes including *Socs3*, which prevents the phosphorylation of STAT3 by JAK, and thus constitutes a negative feedback loop for IL-6 signaling (92, 93). Assessment of STAT3 phosphorylation in mouse muscle was inconclusive under our conditions (not shown), which may occur because STAT3 serves as a target of many signaling pathways (94–96). In this animal model, which was derived from high-fat feeding over time, SOCS3-dependent inhibition of STAT3 phosphorylation precluded conclusions about IL-6 signaling based on the phosphorylation status of STAT3, *i.e.* loss of STAT3 phosphorylation induced by IL-6 receptor activation could indicate decreased IL-6 signaling or, in contrast, increased IL-6 signaling, which would up-regulate SOCS3 and thus inhibit STAT3 phosphorylation. Due to these complicating factors, we utilized *Socs3* expression as a read-out of IL-6 signaling. We found that Socs3 was elevated in muscle in response to high-fat feeding and attenuated in the absence of SphK1.

Muscle-derived IL-6 was proposed to mediate cross-talk between adipose tissue and skeletal muscle during exercise (40,

41); however, the activation of IL-6 signaling, as evidenced by the increase in *Socs3* message in skeletal muscle, but not adipose tissue, suggests that in obesity, this proposed cross-talk may not occur.

Previous studies have demonstrated that *Sphk1* expression is required for the differentiation of myoblasts to myotubes; however, in the absence of SphK1, myogenesis can be induced through the addition of S1P (24). Here, our media for differentiation contained 10% horse serum, and thus, whereas the *Sphk1*$^{-/-}$ myotubes differentiated in the presence of serum, they differentiated at a slower rate than their WT counterparts. The differences in the degree of differentiation were similar to those shown by Meacci *et al.* (24).

The roles of IL-6 and its paracrine signaling in muscle in obesity remain unaddressed in this study; however, data have shown that muscle-derived IL-6 was associated with the induction of satellite cells following muscle damage in humans (97, 98). Thus, we propose that in the context of obesity, myotubes may sense damage due to steatosis, increased oxidative stress, or other metabolic disruptions, which may increase IL-6 production to initiate repair mechanisms, and SphK1 may play a role in this process. This notion is consistent with other studies that suggest roles for SphK1 in muscle regeneration (99). Thus, it is still undetermined whether the function of S1P-induced IL-6 plays a pathological role in this context, or rather, a response to repair muscle damage that may occur in obesity.

S1PR3 is a G-protein-coupled receptor that is expressed in the brain, heart, spleen, liver, lung, kidney, and most important to our study, skeletal muscle (100, 101). Activation of the S1PR3 results in coupling with several G-proteins and the activation of downstream targets including Rho GTPase and MAP kinases (82, 102). Although the involvement of S1PR3 in the production of pro-inflammatory cytokines is not fully understood, it has been implicated in generation of IL-6 in fibroblast-like synoviocytes in rheumatoid arthritis models in response to S1P treatment (102). These studies support the data shown here that S1PR3 plays an important role in S1P-induced IL-6 production in skeletal muscle.

In conclusion, our data demonstrated that in the context of fatty acid oversupply, *Sphk1* is transcriptionally regulated through PPARα, which constitutes a novel mechanism of SphK1 regulation. Moreover, as a PPARα target, SphK1/S1P might be considered a potential effector of signaling by PPARα, a key regulator of whole body lipid metabolism and homeostasis. Additionally, these data indicate that SphK1 plays a mechanistic role in induction of muscle IL-6 in obesity, suggesting that SphK1/S1P mediate some pathophysiological changes associated with obesity. We propose that these data may broaden the known disease contexts in which SphK1/S1P signaling plays roles.

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