

The Peroxisome Proliferator-activated Receptor γ (PPAR γ) Controls Natural Protective Mechanisms against Lipid Peroxidation in Amyotrophic Lateral Sclerosis*[§]

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Background: The mechanism of motor neuron degeneration in amyotrophic lateral sclerosis (ALS) is poorly understood.

Results: The peroxisome proliferator-activated receptor γ (PPAR γ) can be activated by lipid peroxidation metabolites in ALS motor neurons, and this can prompt the expression of antioxidant enzymes.

Conclusion: PPAR γ can exert a direct protective effect in ALS motor neurons.

Significance: PPAR γ transcriptional co-activators may represent therapeutic targets in ALS.

Recent evidence highlights the peroxisome proliferator-activated receptors (PPARs) as critical neuroprotective factors in several neurodegenerative diseases, including amyotrophic lateral sclerosis (ALS). To gain new mechanistic insights into the role of these receptors in the context of ALS, here we investigated how PPAR transcriptional activity varies in hSOD1^{G93A} ALS transgenic mice. We demonstrate that PPAR γ -driven transcription selectively increases in the spinal cord of symptomatic hSOD1^{G93A} mice. This phenomenon correlates with the up-regulation of target genes, such as lipoprotein lipase and glutathione *S*-transferase α -2, which are implicated in scavenging lipid peroxidation by-products. Such events are associated with enhanced PPAR γ immunoreactivity within motor neuronal nuclei. This observation, and the fact that PPAR γ displays increased responsiveness in cultured hSOD1^{G93A} motor neurons, points to a role for this receptor in neutralizing deleterious lipoperoxidation derivatives within the motor cells. Consistently, in both motor neuron-like cultures and animal models, we report that PPAR γ is activated by lipid peroxidation end products, such as 4-hydroxynonenal, whose levels are elevated in the cerebrospinal fluid and spinal cord from ALS patients. We propose that the accumulation of critical concentrations of lipid peroxidation adducts during ALS progression leads to the activation of PPAR γ in motor neurons. This in turn triggers self-protective mechanisms that involve the up-regulation of lipid detoxification enzymes, such as lipoprotein lipase and glutathione *S*-transferase α -2. Our findings indicate that anticipating natural protective reactions by pharmacologically modulating PPAR γ transcriptional activity may atten-

uate neurodegeneration by limiting the damage induced by lipid peroxidation derivatives.

Amyotrophic lateral sclerosis (ALS) is the most common motor neuron disorder of the adult life. In 90–95% of clinical cases the disease occurs with no apparent genetic linkage, whereas in the remaining 5–10% of cases ALS is inherited, usually in an autosomal dominant manner (familial ALS, fALS). Both forms of the disease cause degeneration of motor neurons, leading to paralysis and death within 3–5 years from the appearance of the first symptoms. The discovery that mutations in the gene coding for the enzyme Cu,Zn-superoxide dismutase (SOD1)³ are the most common cause of fALS (20%) prompted the generation of transgenic animal models, which allowed investigations into disease pathogenesis (Refs. 1–4; for review see Ref. 5). Currently, there is no pharmacological intervention or cure to halt the progression of this disorder. The sole FDA approved drug for the treatment of ALS, *i.e.* riluzole, is effective in extending the patient lifespan only by 3–6 months (6). Thus, the identification of novel pharmacological targets and strategies for therapeutic intervention in ALS is highly desirable. A class of signaling molecules, whose relevance for neurodegenerative diseases has become lately evident, is represented by the peroxisome proliferator-activated receptors (PPARs), a family of nuclear receptors that includes three members, *i.e.* PPAR α , $-\beta/\delta$, and $-\gamma$ (7, 8).

In the central nervous system (CNS), PPARs are expressed by neurons as well as glial cells (9), and can be activated by natural lipids and/or their metabolites, including oxidized fatty acids (10). Growing evidence indicates that activation of PPARs, particularly PPAR γ , effectively attenuates neurodegeneration and

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³ The abbreviations used are: SOD, superoxide dismutase; PPAR γ , peroxisome proliferator-activated receptor γ ; PPRE, PPAR responsive element; 4-HNE, 4-hydroxynonenal; Tricine, *N*-[2-hydroxy-1,1-bis(hydroxymethyl)ethyl]glycine; ANOVA, analysis of variance; *Mcad*, medium chain acyl-CoA dehydrogenase; *Acs16*, acyl-CoA synthetase 2; *Lpl*, lipoprotein lipase; *Gsta2*, glutathione *S*-transferase α -2; *Gapdh*, glyceraldehyde-3-phosphate dehydrogenase.

PPAR γ -mediated Lipid Catabolism in ALS

disease progression in animal models of several neurodegenerative disorders (8). However, the specific mechanisms by which these receptors positively affect the course of these diseases have not been elucidated. The beneficial effects of the PPAR γ agonist pioglitazone, described in early pharmacological studies on ALS transgenic mice, were mainly ascribed to the receptor anti-inflammatory activity (11, 12). PPARs can in fact control several aspects of the inflammatory response by repressing the expression of inflammatory genes through interaction with other transcription factors, such as the nuclear factor- κ B and the activator protein 1 (7). However, PPARs can modulate gene expression also directly by binding, as heterodimers with the retinoid X receptors, to specific DNA response elements (PPREs) in the promoter or enhancer regions of target genes. By this means, they exert several homeostatic functions that are crucial for the activity and survival of neural cells (7). Among the functions fulfilled by the PPARs, there is, for example, the capacity to control the expression of enzymes that are critically involved in detoxifying neurons from a wide variety of stress stimuli (13). These PPAR-dependent self-protective mechanisms appear to be relevant for survival of ALS motor neurons. Indeed, expression of PGC-1 α , a transcriptional co-activator of PPAR γ , was recently described to prevent mutant SOD1-induced neuronal cell death *in vitro* (14). Furthermore, both ubiquitous and neuron-specific expression of PGC-1 α was reported to reduce motor neuron degeneration and alleviate the disease outcome in transgenic mice carrying the G93A mutant form of human SOD1 (hSOD1^{G93A}) (15, 16). Altogether, these observations strongly suggest that fine-tuning PPAR transcriptional activity within the CNS may represent an attractive approach to limit the progression of ALS.

To gain further insights into PPAR functions during the progression of the disease, here we bred mice genetically engineered to highlight PPAR transcriptional activity (PPRE-*Luc* mice; Ref. 17) with the hSOD1^{G93A} ALS mouse line (2). We demonstrate that transcriptional activity of PPARs significantly increases in the affected spinal cord at the symptomatic stage of the disease, a phenomenon that takes place in strict temporal and spatial correlation with degeneration of motor neurons. Furthermore, analyses of isoform-specific target genes and cellular distribution indicate that this peculiar process is driven by motor neuronal PPAR γ , possibly through its activation by lipid peroxidation by-products that are generated during the course of the disease.

EXPERIMENTAL PROCEDURES

Transgenic Mice—Transgenic mice expressing the human SOD1^{G93A} (B6SJL-TgN(SOD1-G93A)1Gur) (2) were purchased from The Jackson Laboratories. The colonies were maintained by breeding hemizygote males to wild-type C57Bl6/SJL hybrid females (Charles River Laboratories). Mice genetically engineered to ubiquitously express the *luciferase* reporter gene under control of the PPAR-responsive elements (hereafter referred to as “PPRE-*Luc* mice”) were previously described (17). PPRE-*Luc*^{+/-}; hSOD1^{G93A}^{+/-} double transgenic mice and their PPRE-*Luc*^{+/-}; hSOD1^{G93A}^{-/-} littermates were obtained by cross-breeding hemizygote hSOD1^{G93A} males with homozy-

gous PPRE-*Luc* females. The progeny was screened for the presence of the two transgenes using primers 5'-SOD1 (5'-CATCAGCCCTAATCCATCTGA) and 3'-SOD1 (5'-CGCGACTAACAAATCAAAGTGA) to detect the hSOD1^{G93A} allele, and 5'-PPRE (5'-GGCAGAAGCTATGAAACGAT) and 3'-PPRE (5'-CGACTGAAATCCCTGGTAAT) to amplify the PPRE-*Luc* sequence. Genomic DNA extracted from mouse tail lysates was used as DNA template. To reduce the gender-dependent phenotypic variability described in hSOD1^{G93A} ALS mice (18), only animals of a single sex were analyzed in the present study. Females were selected because they show limited variations in PPAR transcriptional activity upon metabolic and hormonal events (17). Fasting experiments were performed by keeping mice for 48 h in the absence of food.

Intracerebroventricular injection of 4-hydroxynonenal (4-HNE) in anesthetized animals was carried out according to specific stereotaxic coordinates (Bregma, -0.25 mm; lateral, 1 mm; depth, 2.25 mm) using a Hamilton syringe rotated on the coronal plate of about 3 degrees from the orthogonal position, as previously described (19).

All experiments described in this study were conducted according to the Guidelines for Care and Use of Experimental Animals. Use of experimental animals was approved by the Italian Ministry of Research and University and controlled by a panel of experts of the Department of Pharmacological Sciences, University of Milan.

Luciferase Enzymatic Assay—Tissue extracts were prepared by homogenization in 200 μ l of lysis buffer (100 mM KPO₄, 1 mM DTT, 4 mM EGTA, 4 mM EDTA, pH 7.8), one freeze-thaw cycle, and 30 min of centrifugation at 4900 \times g (Rotanta 460R, Hettich Zentrifugen). Supernatants containing luciferase were collected and total protein concentration was determined by the Bradford assay (20). Luciferase enzymatic activity in tissue extracts was then assayed with a luciferase assay buffer (470 μ M luciferine, 20 mM Tricine, 0.1 mM EDTA, 1.07 mM (MgCO₃)₄·Mg(OH)₂ \times 5H₂O; 2.67 mM MgSO₄ \times 7H₂O in H₂O, pH 7.8, with 33.3 mM DTT and 530 μ M ATP). The light intensity was measured with a luminometer (Glomax, Promega) in a 10-s time period and expressed as relative light units/ μ g of protein.

Histological Analysis—Spines were taken and immersed in 4% buffered paraformaldehyde for 24 h. Spinal cords were extracted, the lumbar tract was removed and paraffin embedded. Serial sections of paraffin-embedded spinal cords were cut at 10 μ m and used to perform different immunostainings.

On selected sections, the following immunohistochemical stainings were carried out: PPAR γ (rabbit polyclonal antibody, 1:100; Affinity Bioreagents, Pierce), nonphosphorylated neurofilament H (SMI32, mouse monoclonal antibody, 1:500, Sternberger Monoclonals Inc.), and neuronal nuclei (mouse monoclonal antibody, 1:100, Chemicon) as neuronal markers, and glial fibrillary acidic protein (GFAP, mouse Alexa Fluor 488-conjugated monoclonal antibody, 1:1000, Chemicon) as astrocytic marker. *Licopersicum esculentum* lectin (tomato lectin, 1:200, Sigma) was used to stain microglia. Nuclei were labeled with Hoechst 33342 (Sigma). For quantitative analysis, lumbar spinal cord images (2084 \times 1536 pixels) were captured using a

$\times 40$ objective on a Zeiss Axioskop 40 microscope equipped with a Nikon Coolpix 9900 digital camera.

The number of nuclei positively stained for PPAR γ in the different cell types was determined on images from lumbar spinal cord sections double stained for PPAR γ and SMI32, GFAP, or tomato lectin. The results were expressed as percentage of the total number of cells taken into consideration. The nuclear content of PPAR γ in motor neurons, other neuronal subtypes, and non-neuronal cells was determined by immunofluorescence quantification on images from lumbar spinal cord and brain sections double stained for PPAR γ and SMI32 or neuronal nuclei as follows. Fluorescence intensity of nuclear PPAR γ immunostaining was measured in selected areas using ImageJ software (rsb.info.nih.gov/ij). Fluorescence intensity values were then averaged and expressed relative to mean fluorescence intensity obtained from background noise. Because Hoechst 33342 is a fluorescent dye that intercalates into the DNA, we considered its fluorescence intensity as a measure of the DNA content. Thus, the fluorescence intensity of PPAR γ immunostaining was normalized to the intensity of Hoechst 33342.

For motor neuron quantification, serial sections were stained with 0.5% cresyl violet (Sigma) to detect motor neuron Nissl substance. Motor neurons were counted for a total of 9 disectors using an unbiased stereologic physical disector technique (21).

For morphometric analysis of skeletal muscles, soleus and tibialis anterior (TA) muscles from 30-day-old and 28-month-old C57Bl/6J mice were fixed in 4% paraformaldehyde for 24 h and subsequently embedded in paraffin. Serial sections of paraffin-embedded muscles were cut at 10 μm and stained for hematoxylin and eosin. The minimal "Feret's diameter" was determined using ImageJ software on a minimum of two randomly chosen fields (corresponding to ~ 200 fibers) for each muscle and mouse ($n = 3$).

Plasmid Constructions—The construction of the PPRE $_{5X}$ -tk-Luc plasmid (hereafter referred to as PPRE-Luc transgene) carrying five tandem repeats of PPRE was previously described (17). The bicistronic pSOD1wt-IRES2-ZsGreen1 expression vector coding for both wild-type human SOD1 and the *Zoanthus* sp. green fluorescent protein (ZsGreen) under control of the human cytomegalovirus immediate early promoter was generated by inserting the SOD1 open reading frame (ORF) into the multiple cloning site of the pIRES2-ZsGreen1 vector (Clontech). Briefly, a 465-bp PCR product of the wild-type human SOD1 ORF was prepared by using the pcDNA3-Myc-hSOD1wt plasmid (22) as template and primers F1hSOD1ORFEcoRI (5'-CCGGAAATTCACCA-TGGCGACGAAGGCCGTGTGC) (EcoRI site italicized) and F1hSOD1ORFBamHI (5'-CGCGGATCCTTATTGGG-CGATCCCAATTACAC CACAAG) (BamHI site italicized). The amplicon was cleaved with EcoRI and BamHI and cloned into the EcoRI/BamHI-digested pIRES2-ZsGreen1 backbone to yield plasmid pSOD1wt-IRES2-ZsGreen1. The human mutant SOD1^{G93A} ORF was prepared by PCR using the same primers and the pcDNA3-Myc-hSOD1G93A vector (22) as template. The PCR product was then ligated into the pIRES2-ZsGreen1 vector as described above to yield the

pSOD1G93A-IRES2-ZsGreen1 construct. The entire ORFs were analyzed by sequencing.

Cell Cultures and Transfections—Primary adult neuronal cultures were prepared as previously described (23). Briefly, pups were sacrificed at 5 days of age. Spinal cords were dissected in HABG (HibernatA, 2% B27 supplement, and 0.5 mM glutamine; Invitrogen). Tissues were dissociated with trypsin (1 mg/ml; Sigma) for 30 min at 37 °C. Neurons were purified on OptiPrep density gradient (Sigma), finally resuspended in Neurobasal[®]-A, 2% B27 supplement, 0.5 mM glutamine, 50 units/ml of penicillin, 50 $\mu\text{g}/\text{ml}$ of streptomycin sulfate, 5 ng/ml of FGF, and plated on 0.1 mg/ml of a poly-D-lysine-coated 6-well plate. To remove waste and debris, the medium was changed on day 4 and then every 7 days. Spinal cord mixed glial cell cultures were prepared as previously described, with modifications (22, 24). Mechanical shaking and treatment with the microglial toxin L-leucine methyl ester were omitted to preserve microglial cells and obtain astrocyte-microglia mixed cultures.

NSC-34 (kindly provided by Prof. Neil R. Cashman, University of British Columbia, Vancouver, Canada) is a mouse-mouse hybrid cell line generated by fusion of neuroblastoma cells with motor neuron-enriched embryonic spinal cord cells. This cell line displays several phenotypic traits of motor neurons (25). P0-17D (kindly provided by Prof. James W. Jacobberger, Case Western Reserve University, Cleveland, OH) is a mouse cell line generated by immortalization of neonatal mouse cortical astrocytes with the SV40 large T antigen. Morphologically, these cells resemble mature primary astrocytes (26). BV-2 (kindly provided by Prof. Rosario Donato, University of Perugia, Perugia, Italy) is a murine cell line generated by immortalization of microglial cells with the J2 virus containing both *v-raf* and *v-myc* oncogenes. This cell line exhibits morphological, phenotypic, and functional properties of active microglial cells (27). C2C12 (purchased from Sigma) is a subclone (28) of the mouse myoblast cell line generated by D. Yaffe and O. Saxel (29).

NSC-34, P0-17D, and C2C12 cells were routinely maintained in Dulbecco's modified Eagle's medium (DMEM, Invitrogen) supplemented with 10% fetal bovine serum (FBS, Sigma), 2 mM glutamine, 1 mM sodium pyruvate, and antibiotics (Euroclone). BV-2 cells were cultivated in RPMI 1640 (Invitrogen) supplemented with 10% FBS, 4 mM glutamine, and antibiotics.

Transient transfections of NSC-34, P0-17D, BV-2, and C2C12 cells were performed using FuGENE[®] HD Transfection Reagent (Promega), according to the manufacturer's instructions. Briefly, cells were plated at a density of 2×10^4 cells/well in 24-well plates. The next day, cells were incubated with a FuGENE HD/DNA mixture containing 300 ng of pSOD1wt-IRES2-ZsGreen1 or pSOD1G93A-IRES2-ZsGreen1 vectors, together with 100 ng of PPRE $_{5X}$ -tk-Luc plasmid, which contains the firefly luciferase gene under control of the PPAR-responsive elements (17), and 2 ng of the normalization plasmid pGL4.74 (Promega) containing the *Renilla* luciferase gene. To test the responsiveness of endogenous PPAR γ to pioglitazone, cells were incubated with 30 μM pioglitazone (Alexis Biochemicals) for 24 h and then lysed. To determine the impact of oxidative agents or lipid peroxidation by-products on PPAR transcriptional activity, cells were transfected with 400 ng of

PPAR γ -mediated Lipid Catabolism in ALS

PPRE_{5X}-tk-Luc plasmid and 8 ng of pGL4.74 vector. The next day, cells were exposed to increasing concentrations of hydrogen peroxide (H₂O₂, Sigma) or 4-HNE (Cayman) for 24 h and then lysed. To perform the luciferase enzymatic assay, cells were lysed using a passive lysis buffer (Promega). Firefly and *Renilla* luciferase activities were measured using the Dual-Luciferase Reporter Assay System (Promega) according to the manufacturer's instructions. Firefly luciferase values were normalized to *Renilla* luciferase values and data were reported as relative light units.

RT-PCR Gene Expression Analysis—Spinal cords and hindlimb muscles (composed of gastrocnemius and soleus) were snap-frozen in liquid nitrogen immediately after collection. Tissues were homogenized with a Tissue Lyser (Qiagen) and the RNA was purified using RNeasy Mini Kit or RNeasy Fibrous Tissue Mini Kit (Qiagen) accordingly to the manufacturer's instructions. Total RNA from NSC-34 cells, C2C12 myoblasts, primary neurons, and primary mixed glial cultures was prepared using RNeasy Mini Kit following the manufacturer's instructions for the purification of RNA from animal cells. 1 μ g of tissue- or cell-extracted total RNA was denatured at 75 °C for 5 min in the presence of 1.5 μ g of random primers (Promega) in a 15- μ l final volume. Deoxynucleotide triphosphate (GE Healthcare) and Moloney murine leukemia virus reverse transcriptase (RT) (Promega) were added at 0.5 mM and 8 units/ μ l final concentrations, respectively, in a final volume of 25 μ l. The RT reaction was performed at 37 °C for 1 h; the enzyme was inactivated at 75 °C for 5 min. Control reactions without addition of the RT enzyme were performed for each sample. 1 μ l of the resulting cDNA was analyzed by semiquantitative PCR. The housekeeping gene glyceraldehyde-3-phosphate dehydrogenase (*Gapdh*) was chosen as a reference. Transcripts were detected using primers 5'-GAAGAGTTGGCGTATGGG and 5'-GCGGAGGGCTCTGTACACA for *Mcad*; 5'-GAGGACAGGACAAAGGAGG and 5'-CACGACAAATGCCAACCAAAAAG for *Acs16*; 5'-CACCGGGAGATGAGAGCAA and 5'-CCCAACTCTCATACATTCCC for *Lpl*; 5'-AAGACTGCCTTGCCAAAAGA and 5'-GCCAGTATCTGTGGCTCCAT for *Gsta2*; 5'-ATGCTGGCCTCCCTGATGAAT and 5'-CTGGAGCACCTTGCCGAACA for *Ppar γ* ; 5'-CACCCAAGGGAACCTTGTGCAG and 5'-GGTCGTAGGTGAAGAGAACGG for *Adiponectin*; and 5'-ACGACCCCTTCATTGACC and 5'-TGCTTACCACCTTCTTG for *Gapdh*.

Statistical Analyses—Data are represented as mean \pm S.E. and statistical significance was verified using GraphPad Prism® software. Statistical significance was assessed by *t* test for comparisons between two groups; one-way or two-way ANOVA followed by Bonferroni or Newman-Keuls post hoc tests were used for comparisons of multiple groups.

RESULTS

PPAR Transcriptional Activity Increases in the Spinal Cord of hSOD1^{G93A} ALS Mice at the Symptomatic Stage of the Disease—To analyze the dynamics of PPAR transcriptional activity in hSOD1^{G93A} mice during the progression of the disease, we took advantage of PPRE-*Luc* mice in which the expression of the *luciferase* reporter gene is driven by five tandem

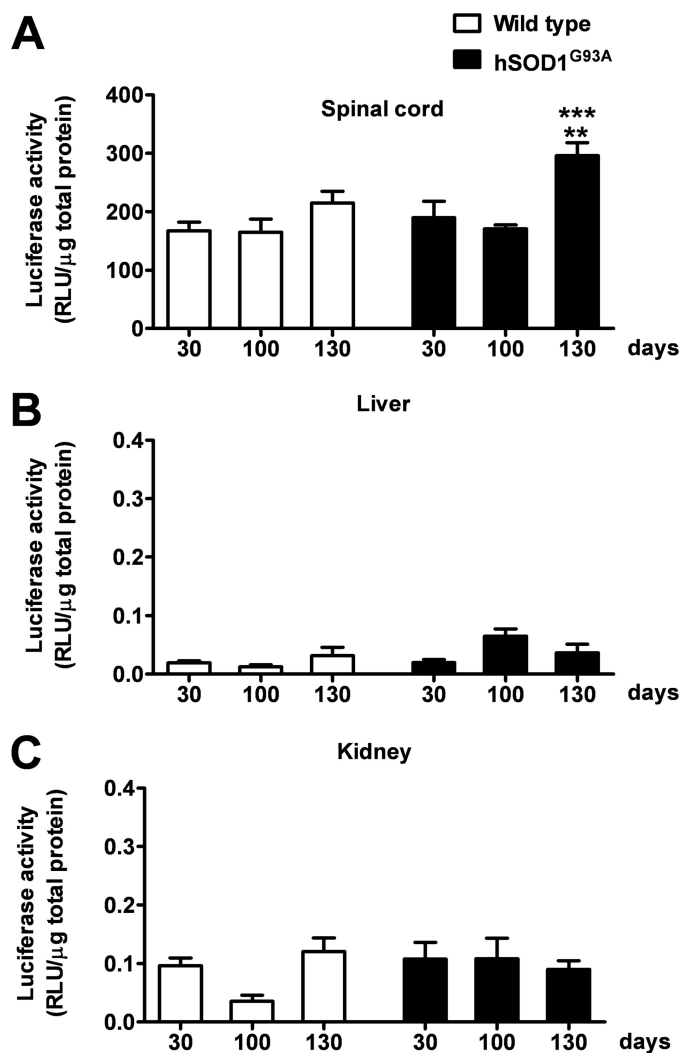


FIGURE 1. PPAR transcriptional activity increases at the symptomatic stage of ALS disease in the spinal cord of hSOD1^{G93A} mice. The spinal cord (A), liver (B), and kidney (C) of PPRE-*Luc*^{+/+}; hSOD1^{G93A}^{-/-} (Wild type) and PPRE-*Luc*^{+/+}; hSOD1^{G93A}^{+/-} (hSOD1^{G93A}) mice were taken at the critical stages of the pathology, i.e. 30 (asymptomatic stage), 100 (onset of motor impairment), and about 130 days of age (symptomatic stage) (*n* = 5–7 mice per time point per genotype). Luciferase enzymatic activity was determined as surrogate of PPAR transcriptional activity on tissue extracts. Data are expressed as mean \pm S.E. **, *p* < 0.01 versus hSOD1^{G93A} at 30 days of age; ***, *p* < 0.001 versus hSOD1^{G93A} at 100 days of age, two-way ANOVA followed by Bonferroni post-hoc test. RLU, relative light units.

repeats of PPRE (17). Homozygous PPRE-*Luc*^{+/+} females were cross-bred with hemizygote hSOD1^{G93A}^{+/-} males to obtain double transgenic PPRE-*Luc*^{+/+}; hSOD1^{G93A}^{+/-} or PPRE-*Luc*^{+/+}; hSOD1^{G93A}^{-/-} mice. Animals of both genotypes were euthanized at the critical stages of ALS disease, i.e. 30 (asymptomatic stage), 100 (onset of motor deficits), and ~130 days (symptomatic stage). The spinal cord, liver, and kidney were then collected and assayed for luciferase enzymatic activity. Data obtained from these analyses indicate that, in PPRE-*Luc*^{+/+}; hSOD1^{G93A}^{+/-} mice, PPAR transcriptional activity is constant in the spinal cord until the onset of the clinical symptoms, but significantly increases at the symptomatic stage of disease progression. By contrast, PPRE-*Luc*^{+/+}; hSOD1^{G93A}^{-/-} animals, which do not express mutant human SOD1, show a fairly constant PPAR transcriptional activity

throughout life (Fig. 1). Interestingly, the aberrant effect identified in the hSOD1^{G93A}-expressing animals is specific for the spinal cord, as it was not observed in peripheral organs, such as liver and kidney, which are not affected by the disease (Fig. 1). Considering that PPARs are involved in several metabolic processes, we next investigated whether the increment in PPAR-

driven luciferase activity identified in symptomatic PPRE-*Luc*^{+/-}; hSOD1^{G93A}^{+/-} mice could be ascribed to alterations in the nutritional state of these animals, due to the oral dysphagia mice experience during the active phase of disease progression (30). Thus, we kept PPRE-*Luc*^{+/-} mice on fasting for 48 h. Animals were then sacrificed and spinal cords were collected and further analyzed for luciferase enzymatic activity. We found that starvation does not influence PPAR transcriptional function in this area of the CNS (Fig. 2), suggesting that the increase in PPAR activity detected at the symptomatic stage of ALS disease strictly depends on the progression of the pathology.

Enhanced PPAR γ -dependent Transcription Correlates with Increased Nuclear Levels of Receptor Selectively in Motor Neurons from Symptomatic ALS Mice—A correlation between the functional data obtained with the reporter luciferase and the specific PPAR isoform implicated in the altered transcriptional process was subsequently addressed by analyzing the expression of isotype-specific endogenous target genes in the spinal cord of hSOD1^{G93A} mice at different ages by semiquantitative RT-PCR. Medium chain acyl-CoA dehydrogenase (*Mcad*) was selected as a endogenous target gene for PPAR α (31), acyl-CoA synthetase 2 (*Acs16*) for PPAR β/δ (32), and lipoprotein lipase (*Lpl*) and glutathione *S*-transferase α -2 (*Gsta2*) for PPAR γ (13, 33) (Fig. 3). Interestingly, we found that only genes whose

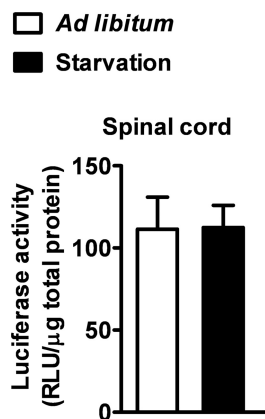


FIGURE 2. Starvation does not increase PPAR transcriptional activity in the mouse spinal cord. PPRE-*Luc*^{+/-} mice were fed *ad libitum* or starved for 48 h. Spinal cords were collected and luciferase enzymatic activity was evaluated ($n = 2$ mice per each condition). Data are expressed as mean \pm S.E. No significant difference was found between the two experimental groups. $p = 0.97$, unpaired *t* test. RLU, relative light units.

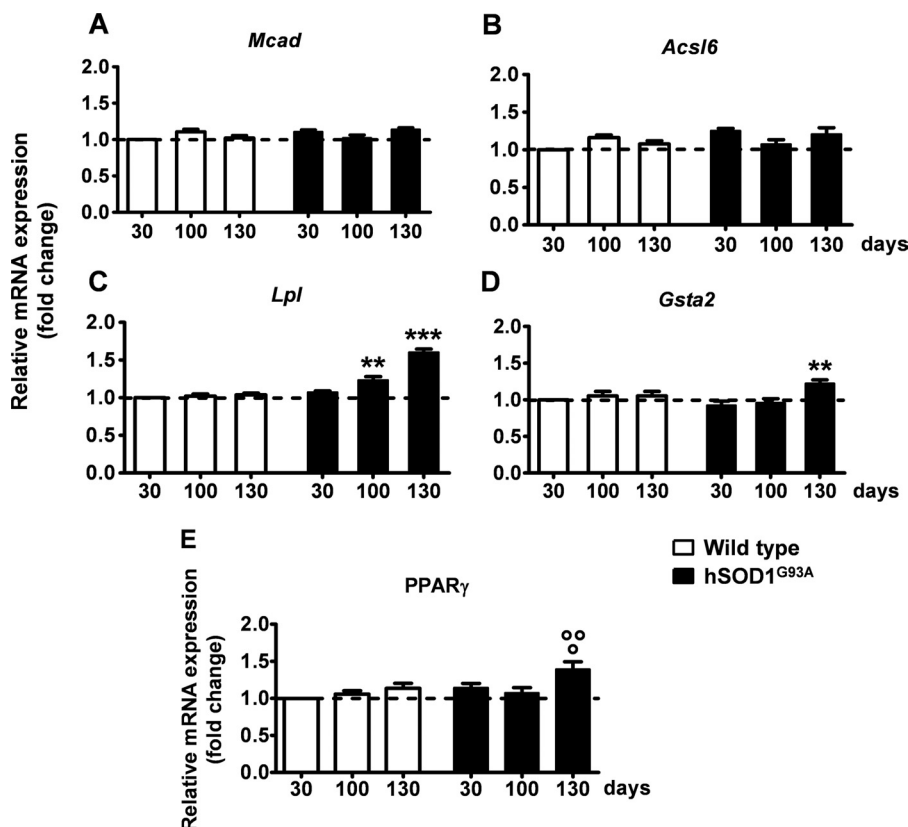


FIGURE 3. Enhanced expression of PPAR γ and its transcriptional target genes in the spinal cord of hSOD1^{G93A} ALS mice at the symptomatic stage of the disease. Expression of PPAR isoform-specific target genes was determined in the spinal cord of wild-type and hSOD1^{G93A} mice of different ages by semi-quantitative RT-PCR analysis ($n = 5$ –7 mice per time point per genotype). *A*, *Mcad* was selected as endogenous target gene for PPAR α ; *B*, *Acs16* for PPAR β/δ ; *C*, *Lpl*; and *D*, *Gsta2* for PPAR γ . *E*, endogenous PPAR γ mRNA was quantified in the spinal cord of wild-type and hSOD1^{G93A} mice ($n = 5$ –7 mice per time point per genotype). Results were expressed as fold-change versus 30-day-old wild-type mice. Data are expressed as mean \pm S.E. **, $p < 0.01$ versus hSOD1^{G93A} at 30 days of age; ***, $p < 0.001$ versus hSOD1^{G93A} at 30 days of age; °, $p < 0.05$ versus hSOD1^{G93A} at 30 days of age; °°, $p < 0.01$ versus hSOD1^{G93A} at 100 days of age, two-way ANOVA followed by Bonferroni post-hoc test.

PPAR γ -mediated Lipid Catabolism in ALS

expression is driven by PPAR γ , such as *Lpl* and *Gsta2*, became significantly up-regulated at the symptomatic stage of ALS progression. By contrast, the messenger RNAs (mRNAs) of the target genes for PPAR α and PPAR β/δ , *Mcad* and *Acsf6*, respectively, did not show significant changes up to 130 days of age, *i.e.* the time when hSOD1^{G93A} mice manifest full-blown symptoms (Fig. 3). This indicates that PPAR γ is the isoform involved in the aberrant transcriptional activity highlighted by the reporter in PPRE-*Luc*^{+/-}; hSOD1^{G93A}^{+/-} spinal cords.

The expression of the receptor itself was subsequently analyzed in the spinal cord of hSOD1^{G93A} mice at different ages during disease progression. Semiquantitative RT-PCR analysis revealed that the PPAR γ mRNA is up-regulated at the symptomatic stage of the disease when compared with the earlier time points and with wild-type animals of the same age (Fig. 3).

Thus, we next decided to investigate the cellular population(s) responsible for the PPAR γ -dependent anomalous transcriptional response. To this aim, we explored the cellular and subcellular distribution of PPAR γ by immunohistochemistry in the spinal cord of hSOD1^{G93A} mice and wild-type littermates at different ages during disease progression. Consistent with previous reports (9), we found that PPAR γ is expressed in motor neurons as well as glial cells, particularly astrocytes and microglia (Fig. 4). To determine whether the enhanced PPAR γ transcriptional activity detected in the spinal cord of aged hSOD1^{G93A} mice could be ascribed to an increase in the number of cells with nuclear PPAR γ , we quantified the proportion of motor neurons, astrocytes, and microglial cells that showed PPAR γ -immunopositive nuclei in the relevant time frame, *i.e.* between 100 and 130 days of age (Fig. 5A). The results obtained from this analysis indicated that 100% of motor neuronal nuclei are immunopositive for PPAR γ throughout life. Besides, the number of astrocytes and microglial cells with PPAR γ -immunopositive nuclei did not vary significantly at the critical time points (astrocytes, 72 \pm 7.9% at 100 days; 58.6 \pm 6.2% at 130 days, p = 0.218, unpaired t test; microglia, 64.2 \pm 13.8% at 100 days; 71.7 \pm 4.4% at 130 days, p = 0.653, unpaired t test). This indicates that the fraction of cells showing PPAR γ into the nucleus remains constant throughout disease progression. Because expression of the receptor increases at the advanced stage of the disease (Fig. 3E), we next postulated that PPAR γ may accumulate within the nucleus of specific cell populations, and this may affect PPAR γ -dependent transcription in such cells. Thus, the nuclear concentration of the receptor was quantitatively investigated on spinal cord sections double labeled for PPAR γ and the neuronal nonphosphorylated neurofilament marker SMI32. Immunofluorescence quantification revealed that the amount of nuclear PPAR γ is significantly higher in motor neurons from symptomatic hSOD1^{G93A} mice when compared with younger animals or age-matched wild-type mice (Fig. 5B). Such an increment in the nuclear concentration of PPAR γ is restricted to motor neurons, because we could not detect a similar increase within spinal cord non-neuronal cells (Fig. 5B) or other neuronal populations present in different areas of the CNS, particularly the cerebral cortex and the hippocampus (Fig. 5C). Thus, we propose that PPAR γ becomes activated in the spinal cord of diseased hSOD1^{G93A} mice, mainly within motor neurons.

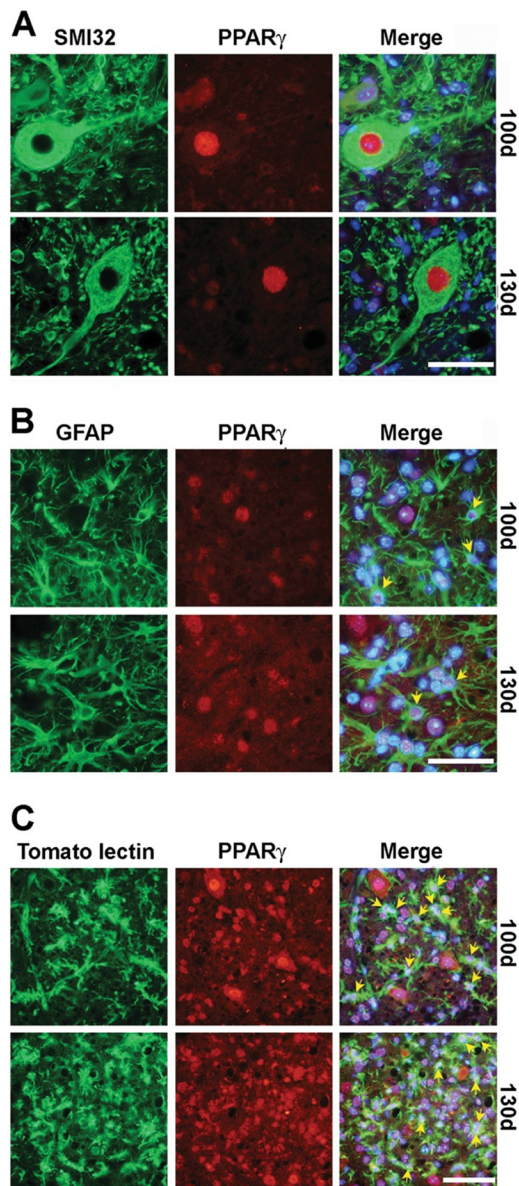


FIGURE 4. Persistent nuclear localization of PPAR γ in both motor neurons and glial cells in the spinal cord of symptomatic hSOD1^{G93A} ALS mice. Double immunofluorescence staining for PPAR γ and SMI32 (A), GFAP (B), or tomato lectin (C) shows intense nuclear staining of PPAR γ in motor neurons, astrocytes, and microglial cells, respectively. Scale bars, 50 μ m.

Skeletal Muscle Atrophy Does Not Affect PPAR γ Expression in Spinal Motor Neurons—One crucial and currently debated issue in ALS is whether alterations within motor neurons are induced retrogradely by the atrophic and degenerative processes muscle fibers undergo during disease progression or whether motor neuronal changes occur cell autonomously (and/or with the contribution of glial cells; for review see Ref. 34) (16, 35–37).

Because considerable evidence indicates that normal aging and ALS share similar structural, regional, and molecular alterations at the level of the neuromuscular system (38), we took advantage of a mouse model of age-related sarcopenia to determine whether the increased expression of PPAR γ within the nuclei of spinal motor neurons might be influenced by alterations of hindlimb myofibers. Numerous reports in fact indicate

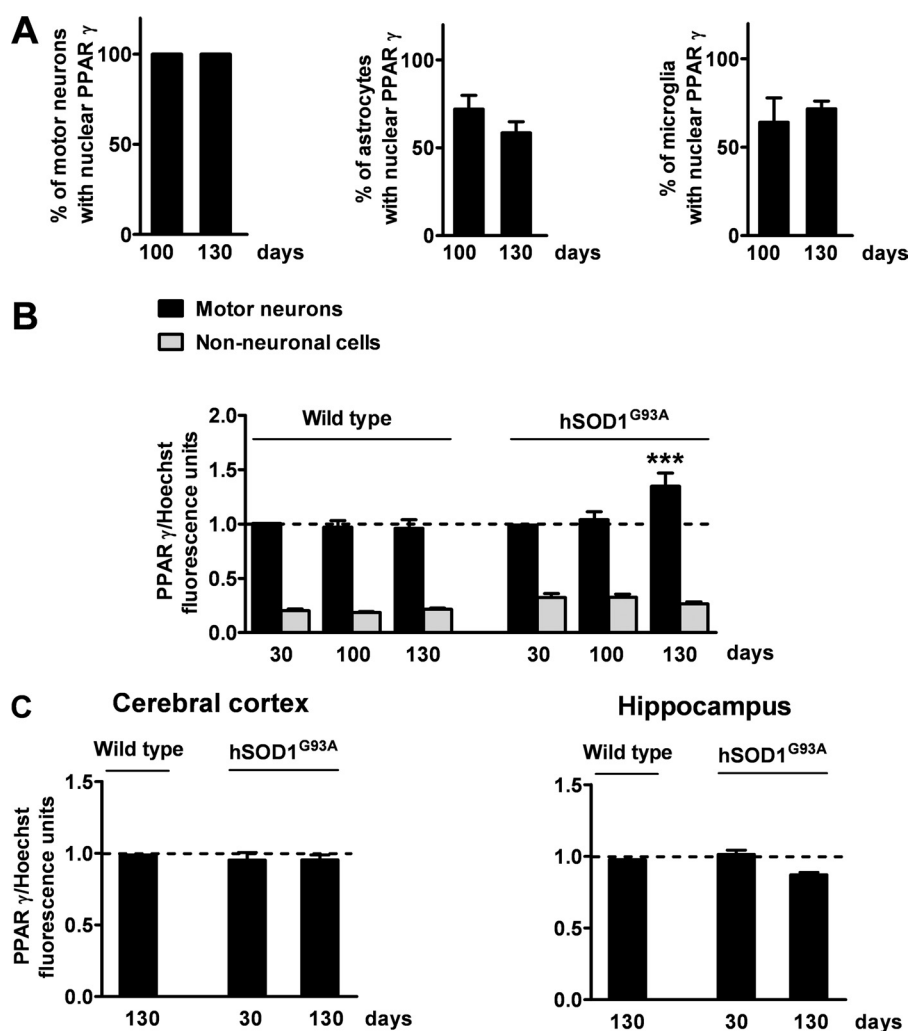


FIGURE 5. The nuclear concentration of PPAR γ is quantitatively enhanced in spinal motor neurons from hSOD1^{G93A} mice at the symptomatic stage of the disease. *A*, the number of motor neurons, astrocytes, or microglial cells showing PPAR γ into the nucleus was determined on spinal cord sections from hSOD1^{G93A} mice taken at 100 (100d) or 130 days of age (130d). Data are expressed as percentage of motor neurons, astrocytes, or microglial cells with nuclear PPAR γ out of the total number of cells taken into consideration ($n = 40$ motor neurons per mouse, $n = 45$ astrocytes per mouse, $n = 100$ microglial cells per mouse; $n = 5-6$ mice per time point). *B*, the intensity of PPAR γ immunofluorescence in the nuclei of motor neurons and non-neuronal cells was determined on lumbar spinal cord sections from wild-type and hSOD1^{G93A} mice stained for PPAR γ , the neuronal marker SMI32 and the nuclear dye Hoechst 33342 ($n = 15$ motor neurons per mouse, $n = 30$ non-neuronal cells per mouse; $n = 3-6$ mice per time point). *C*, the intensity of PPAR γ immunofluorescence in the nuclei of cortical and hippocampal neurons was determined on brain sections from hSOD1^{G93A} mice stained for PPAR γ , the neuronal marker neuronal nuclei and the nuclear dye Hoechst 33342 ($n = 30$ cortical neurons per mouse, $n = 30$ hippocampal neurons per mouse; $n = 3$ mice per time point). Values were normalized relative to fluorescence intensity of Hoechst 33342, as a measure of DNA content. Data are expressed as mean \pm S.E. ***, $p < 0.001$ versus hSOD1^{G93A} motor neurons at 30 and 100 days of age and wild-type at 130 days of age, two-way ANOVA followed by Bonferroni post hoc test.

that, at the age of 28–29 months, C57Bl/6J wild-type mice exhibit a significant atrophy of skeletal muscle that is associated with increased oxidative stress. Noteworthy, this condition is not accompanied by the loss of motor neuronal cell bodies in the lumbar tract of the spinal cord (39–42). Consistent with these observations, we confirmed that 28-month-old C57Bl/6J mice show a significant reduction in the fiber diameter of hindlimb muscles, particularly the soleus and tibialis anterior muscles, when compared with 30-day-old animals of the same strain (Fig. 6A). This state is not associated with a reduction in the number of motor neuronal somas in the lumbar spinal cord (Fig. 6B). Thus, we next determined the concentration of PPAR γ within the nuclei of motor neurons and non-neuronal cells in spinal cord sections from 30-day- and 28-month-old mice double labeled for PPAR γ and SMI32. This analysis revealed that the nuclear levels of the receptor do not vary in

any cell population (Fig. 6C), suggesting that the deleterious changes of skeletal muscle are unlikely responsible for the increased concentration of PPAR γ within the motor neuronal nuclei in the spinal cord of ALS mice.

Motor Neuronal PPAR γ Displays Enhanced Responsiveness in the Presence of Mutant SOD1 or Lipoperoxidative Products in Vitro—To confirm the cell specificity of PPAR γ activation in the different neural cell populations, we switched to the motor neuron-like NSC-34, the astrocytic P0–17D, and the microglial BV-2 cell culture systems (25–27). Cells were transiently co-transfected with the reporter transgene PPRE-Luc together with vectors encoding either the wild-type human SOD1 (hSOD1^{WT}) or the mutant hSOD1^{G93A} proteins. Luciferase activity was then measured 24 h after treating the cells in the absence or presence of the PPAR γ agonist pioglitazone (30 μ M). We found that both the basal and pioglitazone-induced PPAR

PPAR γ -mediated Lipid Catabolism in ALS

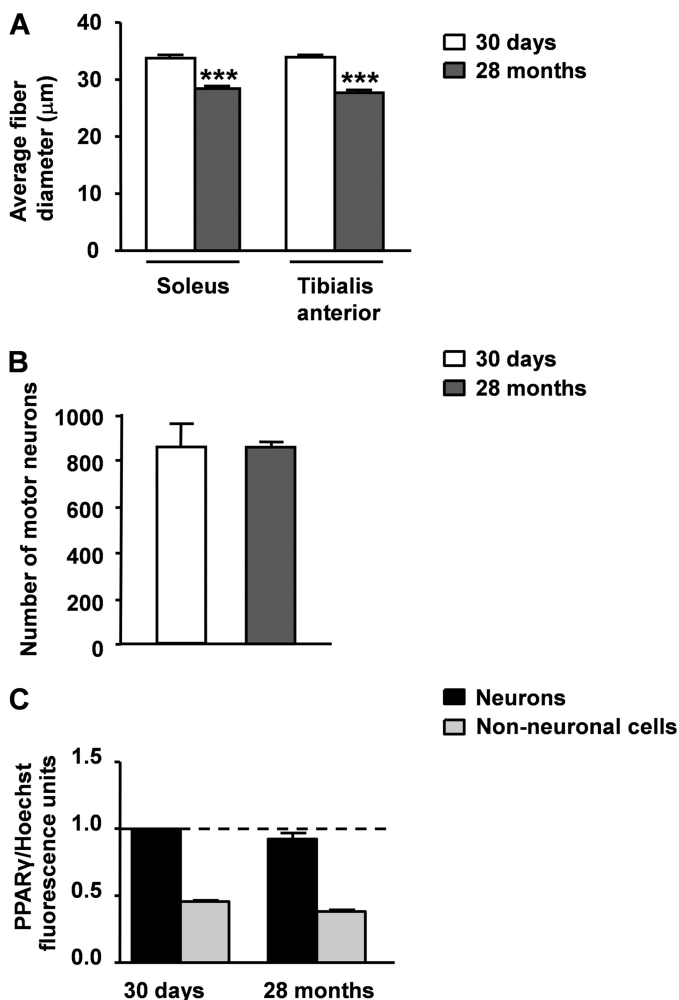


FIGURE 6. Muscle atrophy does not increase the nuclear concentration of PPAR γ within spinal motor neurons. *A*, morphometric analysis of soleus and tibialis anterior muscle fibers from C57Bl/6J wild-type mice at 30 days and 28 months of age. Histograms represent the mean \pm S.E. of the minimal Feret's diameter of the soleus and tibialis anterior muscle fibers ($n = 3$ mice for each age). *B*, quantification of motor neurons in the lumbar tract of the spinal cord from C57Bl/6J mice at 30 days and 28 months of age. Histograms represent the mean \pm S.E. of the total number of motor neurons from 9 dissectors per mouse ($n = 3$ mice for each age). *C*, the intensity of PPAR γ immunofluorescence in the nuclei of motor neurons and non-neuronal cells was determined on lumbar spinal cord sections from 30-day-old and 28-month-old mice stained for PPAR γ , the neuronal marker SMI32 and the nuclear dye Hoechst 33342 ($n = 32$ motor neurons per mouse, $n = 90$ non-neuronal cells per mouse; $n = 3$ mice per time point). Values were normalized relative to fluorescence intensity of Hoechst 33342, as a measure of DNA content. Data are expressed as mean \pm S.E. ***, $p < 0.0001$ versus 30-day-old C57Bl/6J mice, unpaired *t* test.

transcriptional activities are significantly enhanced in the presence of mutant SOD1 in the NSC-34 cells when compared with the wild-type counterpart (Fig. 7A). Interestingly, no such increases were detected in hSOD1^{G93A}-expressing P0-17D and BV-2 glial cell lines, although they resulted in being susceptible to pioglitazone stimulation and PPAR γ activation (Fig. 7A). Consistent with these results, we confirmed an up-regulation of the PPAR γ target genes *Lpl* and *Gsta2* in hSOD1^{G93A}-expressing primary neurons, but not in mixed glial cultures, from mouse spinal cords (Fig. 7, *B* and *C*).

This raised the question as whether the enhanced activity of PPAR γ detected in PPRE-*Luc*^{+/-}; hSOD1^{G93A}+/- mice at the

symptomatic phase of the disease was mainly due to increased expression and sensitivity of the receptor itself, to increased availability of endogenous ligands, or both. To clarify this issue, we reasoned that the symptomatic stage of the disease is characterized by the generation of a plethora of reactive oxygen species in both humans and animal models (43). These agents oxidize various cellular macromolecules, including membrane lipids, and initiate a self-propagating process that results in the production of lipoperoxidation end products, such as 4-HNE and others (44–49). Based on this, we next evaluated the impact of oxidant species and lipid peroxidation by-products, such as H₂O₂ and 4-HNE, respectively, on PPAR-driven luciferase activity *in vitro*. NSC-34 cells were transiently transfected with PPRE-*Luc* and treated with increasing concentrations of H₂O₂ or 4-HNE for 24 h. Luciferase enzymatic activity of cell lysates was then assayed. As shown in Fig. 8, 4-HNE, but not H₂O₂, emerged as a powerful inducer of PPAR transcription. Semiquantitative analysis of the mRNAs coding for the isoform-specific target genes *Mcad*, *Acsl6*, and *Lpl* demonstrated that 4-HNE induces the expression of *Lpl* in a dose-dependent manner (Fig. 9). By contrast, *Mcad* and *Acsl6* expression were not regulated by this agent. These results indicate that PPAR γ is the isoform target for lipid peroxidation end products in motor neuron-like cells.

To determine whether the lipid peroxidation end product 4-HNE is effective in activating PPAR γ transcriptional function also *in vivo*, we intracerebroventricularly injected 4-HNE (5 nmol/mouse) in wild-type mice. The expression of the PPAR γ target genes *Lpl* and *Gsta2* was then analyzed in the spinal cord 3 h later by semiquantitative RT-PCR analysis. Consistent with the data *in vitro*, we confirmed that 4-HNE significantly increases the levels of *Lpl* and *Gsta2* mRNAs (Fig. 10).

PPAR γ Expression and Transcriptional Activity Are Not Increased in Skeletal Muscles from Symptomatic hSOD1^{G93A} ALS Mice—Several lines of evidence indicate that, in both ALS patients and transgenic animal models, skeletal muscles undergo severe atrophy and degeneration, which closely correlate with increased oxidative stress and lipid peroxidation (16, 35–37, 39, 49). To determine whether the occurrence of such events enhanced the expression of PPAR γ in these tissues, we next investigated the levels of the receptor mRNA in hindlimb muscles. Semiquantitative RT-PCR analyses revealed that the expression of PPAR γ transcripts was not significantly different between symptomatic hSOD1^{G93A} mice and age-matched wild-type animals (supplemental Fig. S1A).

Because PPAR γ was consistently reported not to control the expression of scavenger enzymes, such as lipoprotein lipase (LPL), in muscles (50–52), its transcriptional activation was assessed by determining the expression of the tissue-specific PPAR γ target gene *Adiponectin* (52). We found that the levels of *Adiponectin* mRNA did not change between the two genotypes at the age of 130 days (52), indicating that the nuclear receptor is not activated in muscles of symptomatic mice (supplemental Fig. S1A).

To further confirm the impact of mutant SOD1 on PPAR γ transcriptional activity in muscle cells, we transiently co-transfected C2C12 myoblasts with the reporter transgene PPRE-*Luc* and vectors encoding either hSOD1^{WT} or hSOD1^{G93A} proteins. Consist-

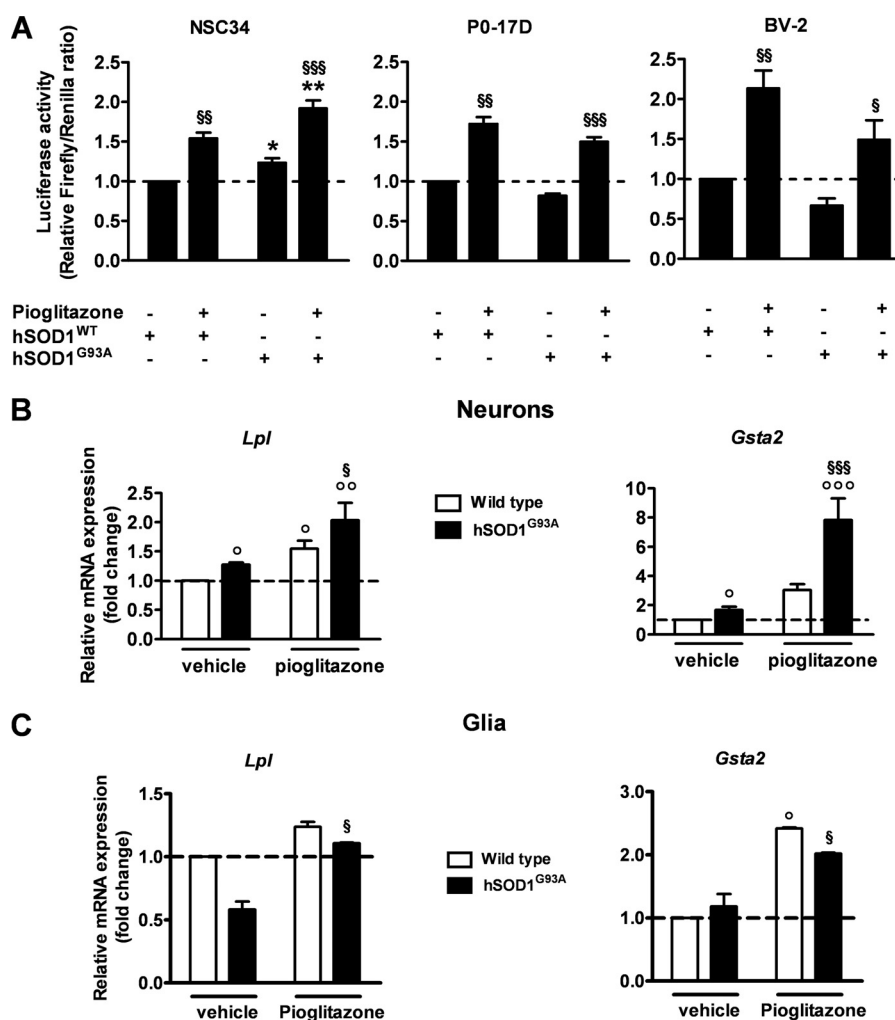


FIGURE 7. Mutant SOD1-expressing neuronal cells are highly responsive to PPAR γ activation *in vitro*. *A*, PPAR transcriptional activity was determined in the motor neuron-like NSC-34, astrocytic P0-17D, and microglial BV-2 cell lines transiently co-transfected with the PPRE_{5x}-tk-Luc plasmid and vectors encoding either hSOD1^{WT} or hSOD1^{G93A}. *Renilla* luciferase was used as a transfection control. Cells were treated in the absence or presence of 30 μ M pioglitazone for 24 h and assayed for luciferase enzymatic activity ($n = 3-6$ in triplicate). Firefly luciferase levels were normalized to *Renilla* luciferase values. *B* and *C*, expression of the PPAR γ target genes *Lpl* and *Gsta2* was determined by semiquantitative RT-PCR analysis in primary neuronal (*B*) and mixed glial cultures (*C*) prepared from the spinal cord of wild-type and hSOD1^{G93A} mice. Cells were treated in the absence and presence of 30 μ M pioglitazone for 24 h ($n = 3$ cultures per cell type). Data are expressed as mean \pm S.E. *, $p < 0.05$ versus hSOD1^{WT}; **, $p < 0.01$ versus hSOD1^{WT} + pioglitazone; §, $p < 0.01$ versus hSOD1^{G93A}; §§, $p < 0.001$ versus hSOD1^{WT}; §§§, $p < 0.001$ versus hSOD1^{G93A}; °, $p < 0.05$ versus wild type; °°, $p < 0.05$ versus wild type + pioglitazone; °°, $p < 0.001$ versus wild type + pioglitazone, two-way ANOVA followed by Bonferroni post-hoc test.

ent with gene expression analyses of skeletal muscles from ALS mice, we did not find significant changes in luciferase activity between the two experimental groups (supplemental Fig. S1B), despite the cells expressed PPAR γ (supplemental Fig. S1C).

This suggests that the presence of mutant SOD1 does not impact the expression and activity of the nuclear receptor in muscle cells *in vivo* and *in vitro*. Thus, we propose that PPAR γ becomes activated in the spinal cord of hSOD1^{G93A} mice to possibly counteract the intense neurodegenerative process that occurs during the symptomatic phase of the pathology.

DISCUSSION

Taking advantage of the PPRE-Luc reporter mouse, which allows to monitor the activity of PPARs during ALS progression, here we show for the first time that PPAR-dependent transcription increases in the spinal cord of hSOD1^{G93A} mice at the

symptomatic stage of the disease. This phenomenon clearly depends on the pathology because it is not observed in peripheral organs, such as kidney and liver, which are unaffected by the disorder.

Immunohistochemical and isoform-specific target gene analyses then led to the identification of motor neuronal PPAR γ as the subtype involved in the aberrant event. Indeed, we show that *Lpl* and *Gsta2*, two genes whose expression is transcriptionally controlled by PPAR γ (13, 33), are up-regulated in the spinal cord of symptomatic hSOD1^{G93A} mice. Our findings are consistent with previous gene expression profiling and proteomic analyses that reported increased levels of LPL and *Gsta2* in both mutant hSOD1^{G93A}-expressing mouse spinal cords and motor neurons in culture (53, 54).

It is noteworthy that, under conditions of oxidative stress, LPL and *Gsta2* were described to limit the propagation of lipid peroxidation events as well as to scavenge cytotoxic lipid

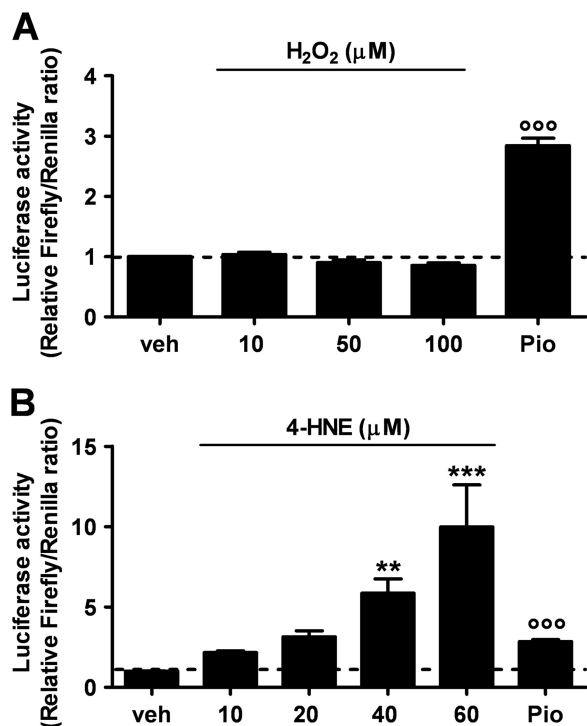


FIGURE 8. The lipid peroxidation adduct 4-HNE, but not the oxidant hydrogen peroxide, triggers PPAR transcriptional activation in cultured motor neuron-like cells. NSC-34 cells were transiently transfected with the PPRE_{5X}-tk-Luc plasmid and treated with increasing concentrations of H₂O₂ (A) or 4-HNE (B) for 24 h ($n = 3$ in triplicate). Pioglitazone (30 μM, Pio) was used as positive control and the vehicle (veh) as negative control. Cell lysates were assayed for luciferase enzymatic activity, and firefly luciferase levels were normalized to the transfection control, *Renilla* luciferase. Data are expressed as mean \pm S.E. ^{ooo}, $p < 0.001$ versus vehicle, H₂O₂, 10, 50, and 100 μM; ^{**}, $p < 0.05$ versus vehicle; ^{***}, $p < 0.01$ versus vehicle, 4-HNE, 10 μM and 20 μM, one-way ANOVA followed by Bonferroni post hoc test.

hydroperoxides *in vitro* (55, 56). This suggests that PPAR γ has the potential to activate defensive mechanisms against lipoperoxidative reactions and related stress-mediated signaling by controlling the expression of antioxidant enzymes.

Remarkably, in tissues where PPAR γ does not control the expression of detoxifying enzymes, such as skeletal muscles (50–52), PPAR γ -dependent transcription does not become activated in ALS mice, providing further support to the idea that the protective function of the receptor is mainly within the nervous system.

Although reports from different laboratories indicate that ALS-driven muscle alterations can impact motor neuronal function and survival (35, 36), others demonstrated that reduction in ALS-dependent muscle atrophy does not prevent neurodegeneration (37). Thus, it is possible that motor neurons and muscle fibers can communicate through specific signaling pathways, but not via others. Herein, we found that the expression of PPAR γ within motor neurons is not influenced by the occurrence of muscle atrophy in aged mice.

Because of the significant oxygen consumption as well as the high content of easily oxidizable polyunsaturated fatty acids, the CNS results particularly vulnerable to oxidative environments. Thus, in several neurodegenerative disorders, including ALS, the formation of lipid oxidation/peroxidation products is a rather common event in all neural tissues that are

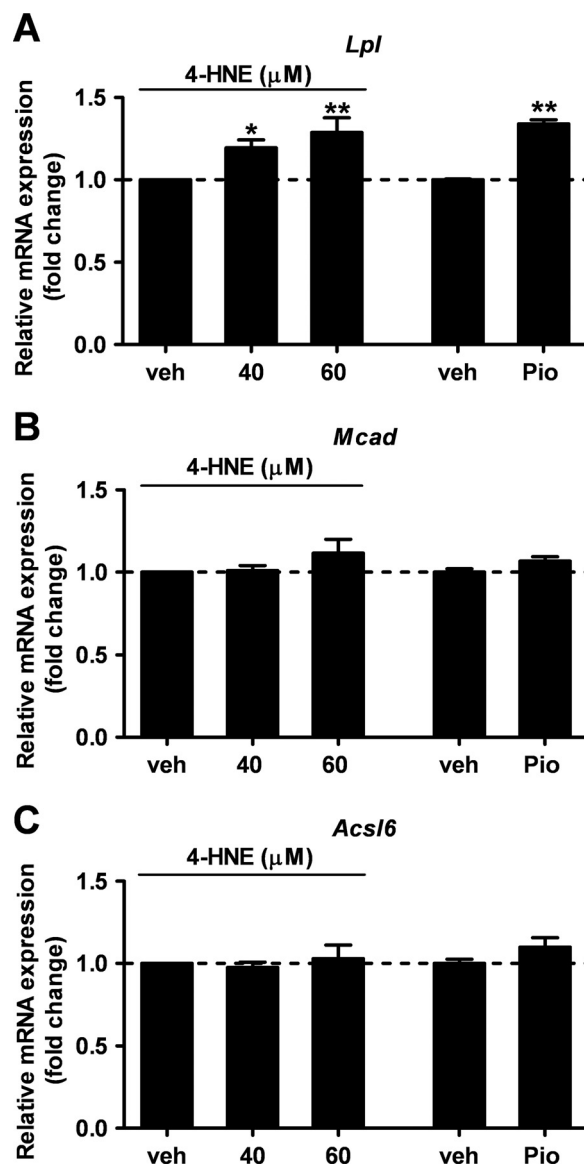


FIGURE 9. 4-HNE specifically enhances the expression of the PPAR γ target gene *Lpl* in cultured motor neuron-like cells. Expression of PPAR isoform-specific target genes, *i.e.* *Lpl* for PPAR γ (A), *Mcad* for PPAR α (B), and *Acs16* for PPAR β/δ (C), was analyzed in NSC-34 cells treated with the vehicle (veh), 4-HNE (40 or 60 μM), or pioglitazone (30 μM) for 24 h ($n = 3$ in triplicate). Data are expressed as mean \pm S.E. ^{*}, $p < 0.05$ versus vehicle; ^{**}, $p < 0.01$ versus vehicle, one-way ANOVA followed by Newman-Keuls post hoc test.

exposed to reactive oxidant species (57). In fact, enhanced levels of malondialdehyde and 4-HNE, two reactive aldehydes that are generated endogenously during the process of peroxidation of membrane fatty acids, have been consistently reported in tissues and spinal fluid from both ALS patients and transgenic animal models (45–49). Although the mechanisms of action of such molecules have not been fully clarified, it is clear that these aldehydic compounds exert their biological effects, at least in part, through reaction with cellular proteins. Hence, previous *in vitro* evidence indicates that 4-HNE impairs glucose and glutamate transport as well as choline acetyltransferase activity in cultured motor neurons, events that precede delayed cell death (58). Furthermore, chronic exposure to high concentrations of 4-HNE was described to trigger motor neuron degeneration *in*

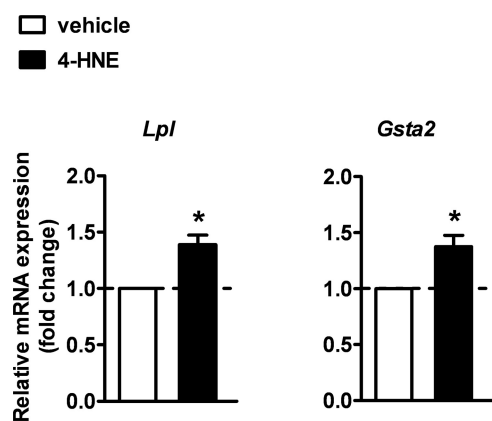


FIGURE 10. 4-HNE enhances the expression of PPAR γ target genes *Lpl* and *Gsta2* *in vivo* in the spinal cord of wild-type mice. Mice were intracerebroventricularly injected with 4-HNE (5 nmol/mouse) or vehicle and the expression of *Lpl* and *Gsta2* was analyzed in the spinal cord after 3 h by semi-quantitative RT-PCR analysis ($n = 4$ –5 mice per each experimental group). Data are expressed as mean \pm S.E. *, $p < 0.05$ versus vehicle, unpaired *t* test.

in vivo (59). Thus, depending on the concentrations and exposure time, the lipid peroxidation end product 4-HNE appears to be causally involved in multiple pathophysiological events both *in vitro* and *in vivo* (60, 61). Because considerable evidence indicates that PPAR γ can be activated by a variety of naturally occurring lipids, including oxidized fatty acids (7, 10), here we investigated the impact of 4-HNE toward the transcriptional activity of PPAR γ . The fact that 4-HNE specifically activated PPAR γ *in vitro*, in motor neuron-like cultures, and *in vivo*, in the spinal cord of wild-type mice, suggests that not only the nuclear receptor interferes with lipoperoxidative processes by controlling the expression of scavenger enzymes, but its activation itself is strongly influenced by lipid peroxidation events and metabolites. Thus, we propose that the accumulation of peroxidized lipids in ALS tissues up to a critical threshold is a key determinant for activation of PPAR γ -dependent self-defensive mechanisms, which are crucial to remove neurotoxic lipid peroxidation by-products. It is worth mentioning that activation of similar natural protective mechanisms is not unusual for the PPARs. In fact, the PPAR β/δ subtype was also reported to be activated by lipid peroxidation products and, thus, critically control physiopathological events in experimental models of other disorders (62).

Further support to the idea that defective lipid metabolism plays a role in ALS progression was provided by a number of clinical and experimental observations. For instance, abnormalities in sphingolipid and cholesterol metabolism were described in the spinal cords of ALS patients and transgenic mouse models, and these changes were reported to sensitize motor neurons to death (63). In addition, decreased lipid storage and increased peripheral lipid clearance were shown in mutant SOD1-expressing mice at the pre-symptomatic stage of the disease (64–66). Consistently, lower serum lipid levels have been more recently related to respiratory impairment in ALS patients (67), and hyperlipidemia was described to be a predictive factor for a better prognosis (68, 69).

Concerning PPAR γ , the implication of this receptor in ALS progression was first suggested by early pharmacological studies on hSOD1^{G93A} mice using the specific agonist pioglitazone

(11, 12). The beneficial effect of this agent was initially ascribed to the anti-inflammatory properties of the receptor. However, the results obtained during the course of this study prompt us to postulate that, besides its anti-inflammatory activity, PPAR γ can also counteract the process of neurodegeneration directly. This occurs through the positive regulation of gene products that limit the accumulation of neurotoxic lipid peroxidation derivatives. Thus, our study points to PPAR γ transcriptional activity as a major player in the defensive response of motor neurons. Furthermore, because treatment with pioglitazone proved effective even when initiated before the onset of the clinical symptoms (11, 12), it is tempting to speculate that anticipating the modulation of PPAR γ transcriptional co-activators pharmacologically may boost these natural protective mechanisms, which normally fail because they become activated only when the disease is already in an advanced stage.

On a therapeutic standpoint, no effective pharmacological intervention to halt the progression of ALS is yet available. The only valuable medication to treat this disorder is currently riluzole, which only slightly slows disease progression. Thus, it seems that further improvement of the outcome of ALS treatments may depend on a better understanding of the molecular mechanisms of motor neuron pathology. In this perspective, and considering our and previous results (11, 12), the transcriptional process driven by PPAR γ emerges as a very interesting mechanism to target with novel therapeutics.

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