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iNOS inhibitor, L-NIL, reverses burn-induced glycogen synthase kinase-3 β activation in skeletal muscle of rats

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Abstract

Objectives—Recent studies suggest that activation of glycogen synthase kinase (GSK)-3 β may be involved in burn injury-induced metabolic derangements and protein breakdown in skeletal muscle. However, the mechanism for GSK-3 β activation after burn injury is unknown. To investigate the role of inducible nitric oxide synthase (iNOS) in this scenario, a major mediator of inflammation, we examined the effects of a specific inhibitor for iNOS, L-NIL, on GSK-3 β activity in skeletal muscle of burned rats.

Materials/Methods—Full-thickness third degree burn injury comprising 40% of total body surface area was produced under anesthesia in male Sprague-Dawley rats (160–190 g) by immersing the back of the trunk for 15 sec and the abdomen for 8 sec in 80°C water. Burned and sham-burned rats were treated with L-NIL (60 mg/kg BW, b.i.d., IP) or phosphate-buffered saline for three days. GSK-3 β activity in skeletal muscle was evaluated by immune complex kinase assay, and by phosphorylation status of GSK-3 β and its endogenous substrate, glycogen synthase.

Results—GSK-3 β activity was increased in a time-dependent manner in skeletal muscle after burn injury, concomitant with the induction of iNOS expression. iNOS inhibitor, L-NIL, reverted the elevated GSK-3 β activity in skeletal muscle of burned rats, although L-NIL did not alter GSK-3 β activity in sham-burned rats.

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Authors' Contributions

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All authors were involved in the manuscript preparation and approved the final version.

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Conclusions—Our results clearly indicate that iNOS plays an important role in burn injury-induced GSK-3 β activation in skeletal muscle. These findings suggest that iNOS may contribute to burn injury-induced metabolic derangements, in part, by activating GSK-3 β .

Keywords

Akt/PKB

1. Introduction

Metabolic derangements following major trauma such as burn injury include catabolism, insulin resistance, and muscle wasting [1,2,3]. Muscle wasting in critically ill patients is a serious clinical issue that results in weaning difficulties from mechanical respirators, prolonged rehabilitation and hospitalization, and worsened prognosis [4,5].

Glycogen synthase kinase (GSK)-3 β phosphorylates and inhibits glycogen synthase (GS) [6]. Akt/PKB inactivates GSK-3 β by phosphorylating serine 9 in GSK-3 β [7]. Activation of GSK-3 β is associated with muscle atrophy, while its inhibition results in hypertrophy of skeletal muscle cells [8]. A previous study indicates that burn injury-induced protein breakdown, a major contributor to muscle wasting, may be ameliorated by *ex vivo* treatment of skeletal muscle with GSK-3 β inhibitors [9]. A recent study has shown that basal GSK-3 β activity is increased in skeletal muscle after burn injury in rats [10]. However, it remains unknown how GSK-3 β is activated following burn injury.

Chronic inflammation has been highlighted as a culprit of obesity-induced insulin resistance [11,12]. We and others have shown that inducible nitric oxide synthase (iNOS), a major mediator of inflammation, plays an important role in obesity-, lipopolysaccharide-, and burn-induced skeletal muscle insulin resistance [13–16]. Inhibition of iNOS improves insulin-stimulated insulin receptor substrate-1-mediated signal transduction in skeletal muscle of obese diabetic mice and burned mice [14,16]. However, the effects of iNOS inhibition on basal (exogenous insulin-naïve) GSK-3 β activity have not yet been investigated in critical illness or obesity. We evaluated the effects of a specific inhibitor for iNOS, L-NIL, on GSK-3 β activity in skeletal muscle of burned rats.

2. Materials and Methods

2.1. Animals

The study protocol was approved by the Institutional Animal Care Committee. The animal care facility is accredited by the Association for Assessment and Accreditation of Laboratory Animal Care. Male Sprague-Dawley rats (160–190 g, Taconic Farms, Germantown, NY) were divided randomly into four groups: sham-burned and burned rats were treated for 3 days with iNOS inhibitor, L-NIL (60 mg/kg BW, b.i.d., IP, Cayman Chemical, Ann Arbor, MI) or phosphate-buffered saline (PBS). A full-thickness third-degree burn injury comprising 40% of total body surface area was produced as described previously [2]. Briefly, rats were treated by immersing the back of the trunk for 15 s and the abdomen for 8 s in 80°C water under anesthesia with pentobarbital sodium (50 mg/kg BW, IP). Sham-

burned rats were immersed in lukewarm water. Buprenorphine (0.05 mg/kg BW, SC) was administered every 8 h for 24 h after burn or sham-burn.

2.2. Tissue Homogenization and Immunoblotting

At 3 days after burn or sham burn, rats were anesthetized with pentobarbital sodium (50 mg/kg BW, IP) following 4-h fasting, and the rectus abdominis muscle was exercised for biochemical analyses. Tissue samples were homogenized as described previously [2]. Immunoblotting was performed as described previously [17]. Anti-Akt1/PKB α , anti-phospho-Akt/PKB (Ser473), anti-GSK-3 β , anti-phospho-GSK-3 β (Ser9) (Cell Signaling, Beverly, MA), anti-GS (Millipore, Billerica, MA), anti-phospho-GS antibodies (Novus Biologicals, Littleton, CA), and anti-glyceraldehyde-3-phosphate dehydrogenase (GAPDH) (Trevigen, Gaithersburg, MD) antibodies were used as primary antibodies. Bands of interest were scanned with the HP Scanjet 4850 (Hewlett-Packard, Palo Alto, CA) and quantified by NIH Image 1.62 software (NTIS, Springfield, VA).

2.3. Immunohistochemistry

Muscle cryosections were stained for iNOS (1:50 dilution, Millipore) and caveolin-3 (1:30 dilution, BD Biosciences, San Jose, CA). The latter is specifically expressed in skeletal, cardiac, and smooth muscle cells [18]. The stained microsections were then visualized with goat anti-mouse and goat anti-rabbit Ig [F(ab')₂] conjugated with Texas Red and FITC (Jackson ImmunoResearch, West Grove, PA) (1:50 dilution) using a Nikon Eclipse TE 2000-S fluorescent microscope (Nikon, Melville, NY).

2.4. GSK-3 β Kinase Assay

Immunoprecipitates with anti-GSK-3 β antibody (BD Biosciences, Franklin Lakes, NJ) were incubated in kinase buffer (50 mM HEPES, pH 7.4, 10 mM MgCl₂, 10 mM MnCl₂, 0.1 mM CaCl₂, and 0.1 mM sodium vanadate) in the presence of ATP (100 μ M) and [γ -³²P]ATP (0.5 μ Ci/sample) for 5 min at 30°C. For substrate, we used a peptide (YRRAAVPPSPSLSRHSSPHQSEDEEE, Millipore) that corresponds to the amino acid sequence of the GSK-3 β phosphorylation site in GS.

2.5. Measurements of Glycogen Synthase Activity, Glycogen Content, and Nitrotyrosine

Glycogen synthase activity was measured as previously described [19]. Briefly, 30 μ l of homogenate was added to 30 μ l of assay buffer containing 50 mM Tris-HCl pH 7.4, 50 mM NaF, 10 mM EDTA, 10 mM UDP-glucose, 1.5 μ Ci/ml [¹⁴C]UDP-glucose (Amersham), and 15 mg/ml glycogen. After 15-min incubation at 37°C, incorporation of ¹⁴C radioactivity in glycogen was measured with the liquid scintillation counter. Glycogen content in muscle was measured as previously described [20] using purified glycogen (Sigma, St. Louis, MO) as a standard. Nitrotyrosine content was measured using ELISA kit (Cell Biolabs, San Diego, CA) according to the manufacturer's instruction.

2.6. Statistical Analysis

The data were compared with one-way ANOVA followed by Newman-Keuls multiple comparison test. A value of $P < 0.05$ was considered statistically significant. All values are expressed as means $\pm$ SEM.

3. Results

3.1. Increased GSK-3 β activity paralleled iNOS induction in skeletal muscle after burn injury

Phosphorylation of GSK-3 β at serine 9 decreased in a time-dependent manner starting 1 day after burn compared with control rats (Fig. 1A, B). Consistently, phosphorylation of an endogenous substrate of GSK-3 β , GS, increased after burn. In contrast, GS protein expression was significantly decreased at 1, 2, 3, and 7 days after burn injury, while GSK-3 β and GAPDH protein expression were not altered by burn. It should be noted that phosphorylation of GS significantly increased regardless of whether it was normalized to GS expression (p-GS/GS) or not (p-GS) (Fig. 1B, middle panel).

Akt/PKB inhibits GSK-3 β activity by phosphorylating serine 9 in GSK-3 β [7]. Therefore, we assessed Akt/PKB activity by the phosphorylation status of Akt/PKB. Similar to GSK-3 β phosphorylation, phosphorylation of Akt/PKB was decreased after burn injury in a time-dependent manner (Fig. 1A, B). Akt/PKB protein expression was not altered by burn.

iNOS protein expression was induced in skeletal muscle by burn in a time-dependent manner (Fig. 1A, B). The immunohistochemical analysis revealed that iNOS was expressed in muscle cells, which were positive for caveolin-3, a muscle-specific marker [18], after burn (Fig. 1C). Without burn injury, however, iNOS was not detected in skeletal muscle by immunoblotting or immunohistochemistry.

3.2. iNOS inhibitor, L-NIL, reversed GSK-3 β activation in skeletal muscle of burned rats

We treated burned and sham-burned rats with L-NIL or PBS for 3 days. The burn-induced iNOS expression was observed in both iNOS inhibitor- and PBS-treated animals to a similar extent (Fig. 2A, B). L-NIL reversed the burn-induced decreased inhibitory GSK-3 β phosphorylation and increased GSK-3 β activity compared with PBS. GSK-3 β protein expression was not altered by burn injury or iNOS inhibitor. Phosphorylation of GS was elevated in muscle of burned rats compared with sham-burn, which was reversed by L-NIL. GS protein expression was decreased by burn injury, and L-NIL restored decreased GS expression in burned rats. GS activity and glycogen content were decreased in skeletal muscle of PBS-treated burned rats compared with sham-burn (Fig. 2B), both of which were restored by L-NIL.

Akt/PKB phosphorylation was decreased in burned rats relative to sham-burn (Fig. 2A, B). L-NIL restored the suppressed Akt/PKB phosphorylation in burned rats. Akt/PKB protein expression was not altered by burn injury or L-NIL. To assess the effects of L-NIL on iNOS activity *in vivo*, we measured nitrotyrosine content in skeletal muscle. Burn injury significantly increased nitrotyrosine content, which was reversed by L-NIL (Fig. 2B).

4. Discussion

Here, we demonstrate that basal GSK-3 β activity is increased in skeletal muscle following burn in rats, as judged by phosphorylation of GSK-3 β at serine 9, immune complex kinase assay, and the phosphorylation status of GS, an endogenous substrate of GSK-3 β . Moreover, our data show that: (1) elevated GSK-3 β activity following burn parallels iNOS induction (Fig. 1); and (2) iNOS inhibitor, L-NIL, reverts the increased GSK-3 β activity in skeletal muscle of burned rats (Fig. 2). These results clearly indicate that iNOS plays an important role in burn-induced activation of GSK-3 β in skeletal muscle.

This finding of burn-induced activation of GSK-3 β in skeletal muscle is in agreement with a recent study [10]. Fang et al. showed that basal GSK-3 β activity was increased in skeletal muscle at 8 h and 24 h after burn, but they did not evaluate GSK-3 β activity at later time points [10]. We found that increased GSK-3 β activity peaks at 3 days and this activity is sustained up to at least 7 days after burn.

Increased GSK-3 β activity in burned rats was associated with elevated GS phosphorylation and decreased GS activity, both of which were restored to control levels by iNOS inhibitor (Fig. 2). In contrast to increased phosphorylation of GS by burn injury, the protein expression of GS was decreased in burned rats compared to control animals. The decreased GS expression in burned rats was restored by iNOS inhibitor, in parallel with the reversal of increases in GSK-3 β activity and GS phosphorylation. Notably, a previous study has demonstrated that constitutive activation of GSK-3 β downregulates the abundance of GS in rat skeletal muscle cells [21]. One can speculate, therefore, that GSK-3 β activation and its reversal might be involved in the decreased GS expression in burned rats and its restoration by iNOS inhibitor.

Previously we have shown that burn impairs insulin-stimulated Akt/PKB phosphorylation (activation) in rats [2] and mice [16] after overnight fasting. In the present study, we observed that basal (exogenous insulin-naïve) phosphorylation of Akt/PKB was decreased in skeletal muscle of burned rats compared with the sham-burn controls following 4-h fasting (Fig. 2). Decreased basal Akt/PKB phosphorylation was also reversed by iNOS inhibitor. Thus, these findings suggest that burn injury-induced decreased basal activity of Akt/PKB, which is mediated by iNOS, may contribute to increased GSK-3 β activity in burned rats via hypophosphorylation of serine 9 in GSK-3 β (Fig. 2C). Our results are in line with a recent study that lipid infusion decreases basal Akt/PKB phosphorylation in skeletal muscle of wild-type, but not iNOS knockout, mice [22]. Based on previous studies [17, 20], one can speculate that nitrosative protein modifications, such as S-nitrosylation and tyrosine nitration, may be possibly involved in iNOS-mediated decreases in phosphorylation of Akt/PKB. Further studies are required to clarify the precise mechanisms underlying iNOS-mediated alterations in Akt/PKB and GSK-3 β activities after burn injury.

In summary, our data indicate that iNOS plays an important role in burn injury-induced GSK-3 β activation in skeletal muscle. It is conceivable that iNOS-mediated GSK-3 β activation may be involved in burn-induced muscle wasting and metabolic derangements.

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Abbreviations

GS	glycogen synthase
GSK-3β	glycogen synthase kinase-3 β
iNOS	inducible nitric-oxide synthase
L-NIL	N ⁶ -(1-iminoethyl)-l-lysine
PBS	phosphate-buffered saline

References

1. Cree MG, Wolfe RR. Post Burn Trauma Insulin Resistance and Fat Metabolism. *Am J Physiol Endocrinol Metab.* 2008; 294:E1–9. [PubMed: 17957035]
2. Sugita H, Kaneki M, Sugita M, Yasukawa T, Yasuhara S, Martyn JA. Burn injury impairs insulin-stimulated Akt/PKB activation in skeletal muscle. *Am J Physiol Endocrinol Metab.* 2005; 288:E585–591. [PubMed: 15536206]
3. Yu YM, Tompkins RG, Ryan CM, Young VR. The metabolic basis of the increase of the increase in energy expenditure in severely burned patients. *JPEN.* 1999; 23:160–168.
4. Friedrich O. Critical illness myopathy: what is happening? *Curr Opin Clin Nutr Metab Care.* 2006; 9:403–409. [PubMed: 16778569]
5. Schweickert WD, Hall J. ICU-acquired weakness. *Chest.* 2007; 131:1541–1549. [PubMed: 17494803]
6. Rylatt DB, Aitken A, Bilham T, Condon GD, Embi N, Cohen P. Glycogen synthase from rabbit skeletal muscle. Amino acid sequence at the sites phosphorylated by glycogen synthase kinase-3, and extension of the N-terminal sequence containing the site phosphorylated by phosphorylase kinase. *Eur J Biochem.* 1980; 107:529–537. [PubMed: 6772446]
7. Summers SA, Kao AW, Kohn AD, Backus GS, Roth RA, Pessin JE, Birnbaum MJ. The role of glycogen synthase kinase 3 β in insulin-stimulated glucose metabolism. *J Biol Chem.* 1999; 274:17934–17940. [PubMed: 10364240]
8. Rommel C, Bodine SC, Clarke BA, Rossman R, Nunez L, Stitt TN, Yancopoulos GD, Glass DJ. Mediation of IGF-1-induced skeletal myotube hypertrophy by PI(3)K/Akt/mTOR and PI(3)K/Akt/GSK3 pathways. *Nat Cell Biol.* 2001; 3:1009–1013. [PubMed: 11715022]
9. Fang CH, Li BG, James JH, King JK, Evenson AR, Warden GD, Hasselgren PO. Protein breakdown in muscle from burned rats is blocked by insulin-like growth factor I and glycogen synthase kinase-3 β inhibitors. *Endocrinology.* 2005; 146:3141–3149. [PubMed: 15802492]
10. Fang CH, Li B, James JH, Yahya A, Kadeer N, Guo X, Xiao C, Supp DM, Kagan RJ, Hasselgren PO, Sheriff S. GSK-3 β activity is increased in skeletal muscle after burn injury in rats. *Am J Physiol Regul Integr Comp Physiol.* 2007; 293:R1545–1551. [PubMed: 17686886]
11. Kim JK, Kim YJ, Fillmore JJ, Chen Y, Moore I, Lee J, Yuan M, Li ZW, Karin M, Perret P, Shoelson SE, Shulman GI. Prevention of fat-induced insulin resistance by salicylate. *J Clin Invest.* 2001; 108:437–446. [PubMed: 11489937]
12. Martyn JA, Kaneki M, Yasuhara S. Obesity-induced insulin resistance and hyperglycemia: etiologic factors and molecular mechanisms. *Anesthesiology.* 2008; 109:137–148. [PubMed: 18580184]

13. Perreault M, Marette A. Targeted disruption of inducible nitric oxide synthase protects against obesity-linked insulin resistance in muscle. *Nat Med*. 2001; 7:1138–1143. [PubMed: 11590438]
14. Sugita H, Fujimoto M, Yasukawa T, Shimizu N, Sugita M, Yasuhara S, Martyn JA, Kaneki M. Inducible nitric-oxide synthase and NO donor induce insulin receptor substrate-1 degradation in skeletal muscle cells. *J Biol Chem*. 2005; 280:14203–14211. [PubMed: 15805118]
15. Carvalho-Filho MA, Ueno M, Carvalheira JB, Velloso LA, Saad MJ. Targeted disruption of iNOS prevents LPS-induced S-nitrosation of IRbeta/IRS-1 and Akt and insulin resistance in muscle of mice. *Am J Physiol Endocrinol Metab*. 2006; 291:E476–482. [PubMed: 16638822]
16. Sugita M, Sugita H, Kim M, Mao J, Yasuda Y, Habiro M, Shinozaki S, Yasuhara S, Shimizu N, Martyn JA, Kaneki M. Inducible nitric oxide synthase deficiency ameliorates skeletal muscle insulin resistance but does not alter unexpected lower blood glucose levels after burn injury in C57BL/6 mice. *Metabolism*. 2012; 61(1):127–36. [PubMed: 21816442]
17. Yasukawa T, Tokunaga E, Ota H, Sugita H, Martyn JA, Kaneki M. S-nitrosylation-dependent inactivation of Akt/protein kinase B in insulin resistance. *J Biol Chem*. 2005; 280:7511–7518. [PubMed: 15632167]
18. Song KS, Scherer PE, Tang Z, Okamoto T, Li S, Chafel M, Chu C, Kohtz DS, Lisanti MP. Expression of caveolin-3 in skeletal, cardiac, and smooth muscle cells. Caveolin-3 is a component of the sarcolemma and co-fractionates with dystrophin and dystrophin-associated glycoproteins. *J Biol Chem*. 1996; 271:15160–15165. [PubMed: 8663016]
19. Thomas JA, Schlender KK, Lerner J. A rapid filter paper assay for UDPglucose-glycogen glucosyltransferase, including an improved biosynthesis of UDP-14C-glucose. *Anal Biochem*. 1968; 25:486–499. [PubMed: 5704765]
20. Shinozaki S, Choi CS, Shimizu N, Yamada M, Kim M, Zhang T, Shiota G, Dong HH, Kim YB, Kaneki M. Liver-specific inducible nitric-oxide synthase expression is sufficient to cause hepatic insulin resistance and mild hyperglycemia in mice. *J Biol Chem*. 2011; 286:34959–34975. [PubMed: 21846719]
21. MacAulay K, Blair AS, Hajduch E, Terashima T, Baba O, Sutherland C, Hundal HS. Constitutive activation of GSK3 down-regulates glycogen synthase abundance and glycogen deposition in rat skeletal muscle cells. *J Biol Chem*. 2005; 280:9509–9518. [PubMed: 15632169]
22. Cha HN, Song SE, Kim YW, Kim JY, Won KC, Park SY. Lack of inducible nitric oxide synthase prevents lipid-induced skeletal muscle insulin resistance without attenuating cytokine level. *J Pharmacol Sci*. 2011; 117:77–86. [PubMed: 22001626]

Fig. 1.

Time-dependent GSK-3 β activation paralleled iNOS induction in rat skeletal muscle after burn injury. A, B, Immunoblotting revealed that phosphorylation of GSK-3 β at serine 9 (p-GSK-3 β) was decreased in muscle after burn injury, while phosphorylation of GS (xp-GS) was increased. GSK-3 β and GAPDH protein expression were not altered by burn injury. In contrast, GS protein expression was decreased by burn injury. These findings indicate that GSK-3 β activity was increased after burn injury. Similar to phosphorylation of GSK-3 β , Akt/PKB phosphorylation was decreased after burn injury in a time-dependent manner, while Akt/PKB protein expression was not altered by burn injury. iNOS protein expression was induced after burn injury. The maximum changes in phosphorylation of GSK-3 β , GS and Akt/PKB and in the maximum iNOS induction were concomitantly observed at 3 days after burn injury. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ vs. before burn injury, $n = 4$ for each time point. C, Immunohistochemical analysis revealed that iNOS was expressed in caveolin-3-positive skeletal muscle cells of burned, but not sham-burned, rats.

Fig. 2. iNOS inhibitor, L-NIL, reversed burn injury-induced GSK-3β activation in rat skeletal muscle. Burned and sham-burned rats were treated with L-NIL (60 mg/kg BW, b.i.d., IP) or PBS for 3 days. A, B, Immunoblotting showed that iNOS expression was induced at 3 days after burn injury, while iNOS was not detected in sham-burned rats. iNOS inhibitor, L-NIL, did not alter iNOS expression, but reversed iNOS-associated increased nitrotyrosine content in burned rats. Burn injury decreased inhibitory phosphorylation of GSK-3β at serine 9 (p-GSK-3β) compared with sham-burn when treated with PBS. iNOS inhibitor, L-NIL, restored decreased phosphorylation of GSK-3β in burned rats. Consistently, immune complex kinase assay showed that burn injury increased GSK-3β activity in PBS-treated rats compared with sham-burn, which was reversed by L-NIL. Inhibitory phosphorylation of GS (p-GS) was increased and GS expression was decreased after burn injury in PBS-treated rats, and L-NIL reversed these changes in burned rats. Burn significantly decreased GS activity and glycogen content in PBS-treated rats as compared with sham animals, both of which were reversed or mitigated by L-NIL. Consistent with the decreased GSK-3β phosphorylation, Akt phosphorylation (p-Akt) was decreased by burn injury in PBS-treated rats, and L-NIL treatment restored Akt phosphorylation in burned rats to the levels observed in sham animals. GSK-3β, Akt, and GAPDH protein expression were not altered by burn injury or L-NIL. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ vs. sham and L-NIL-treated animals, ## $p < 0.01$ vs. sham animals, N.S.: no significant difference. $n = 4$ for each group of sham animals, $n = 8$ for each group of burned animals. C, A potential iNOS-mediated mechanism by which burn injury activates GSK-3β in rat skeletal muscle.