Hindawi Publishing Corporation
International Journal of Photoenergy
Volume 2014, Article ID 384816, 8 pages
http://dx.doi.org/10.1155/2014/384816

Research Article

Photobiomodulation on Bax and Bcl-2 Proteins and SIRT1/PGC-1α Axis mRNA Expression Levels of Aging Rat Skeletal Muscle

Fang-Hui Li,[1,2] Yan-Ying Liu,[1] Fei Qin,[1] Qing Luo,[3] Hai-Ping Yang,[2] Quan-Guang Zhang,[4] and Timon Cheng-Yi Liu[1]

[1] *Laboratory of Laser Sports Medicine, South China Normal University, University Town, Guangzhou 510006, China*
[2] *School of Physical Education and Health, Zhaoqing University, Zhaoqing 526061, China*
[3] *Teaching Department of Ideological and Political Theory, Zhaoqing University, Zhaoqing 526061, China*
[4] *Institute of Molecular Medicine and Genetics, Medical College of Georgia at Georgia Regents University, Augusta, GA 30912, USA*

Correspondence should be addressed to Timon Cheng-Yi Liu; liutcy@scnu.edu.cn

Received 27 February 2014; Revised 23 May 2014; Accepted 24 May 2014; Published 19 June 2014

Academic Editor: Hongtao Yu

Objective. This study aimed to analyze the effects of low level laser irradiation (LLLI) on Bax and IGF-1 and Bcl-2 protein contents and SIRT1/PGC-1α axis mRNA expression levels to prevent sarcopenia in aged rats. *Material and Methods*. Twenty female Sprague Dawley rats (18 months old) were randomly divided into two groups ($n = 10$ per group): control (CON) and LLLI groups. The gallium-aluminum-arsenium (GaAlAs) laser irradiation at 810 nm was used in the single point contact mode (3.75 J/cm^2; 0.4 cm^2; 125 mW/cm^2; 30 s). Bax, Bcl-2, and IGF-1 proteins and SIRT1/PGC-1α axis mRNA expression were assessed 24 h after LLLI on gastrocnemius in aged rat. *Results*. Gastrocnemius muscle weights, gastrocnemius mass/body mass, Bcl-2/BAX ratio, Bcl-2 protein, IGF-1 protein, and the mRNA contents in SIRT1, PGC-1α, NRF1, TMF, and SOD2 were significantly ($P < 0.05$) increased by LLLI compared to CON group without LLLI. However, levels of BAX protein and caspase 3 mRNA were significantly attenuated by LLLI compared to CON group ($P < 0.05$). *Conclusion*. LLLI at 810 nm inhibits sarcopenia associated with upregulation of Bcl-2/BAX ratio and IGF-1 and SIRT1/PGC-1α axis mRNA expression in aged rats. This indicates that LLLI has potential to decrease progression of myocyte apoptosis in sarcopenic muscles.

1. Introduction

Sarcopenia, the age-related decline in muscle mass and function, represents a significant health issue due to its associated high prevalence of frailty and disability [1]. An altered regulation of myocyte apoptosis has recently emerged as a possible contributor to the pathogenesis of sarcopenia [2]. Studies in animal models [2] and human [3] have shown that the severity of skeletal muscle cell apoptosis increases over the course of aging and correlates with the degree of muscle mass and strength decline. Several apoptotic pathways are operative in aged muscles, with the mitochondria-mediated pathways being the most likely relevant to sarcopenia [4].

Sirtuin 1 (SIRT1), a nicotinamide adenine dinucleotide-(NAD^+-) dependent histone deacetylase [5], is implicated in the prevention of many age-related diseases such as sarcopenia, cancer, Alzheimer's disease, and type 2 diabetes, by maintaining mitochondrial homeostasis [6]. SIRT1/PGC-1α (peroxisome proliferator-activated receptor-γ coactivator 1α) pathway plays a vital role in regulating mitochondria oxidative stress, biogenesis, and apoptosis, thereby contributing to maintain skeletal muscle homeostasis and human longevity [5]. Therefore, disruption of SIRT1/PGC-1α pathway may result in age-related loss of muscle function and sarcopenia. Indeed, muscle wasting could be prevented by reducing myocyte apoptosis and proteasome degradation if the transcriptional activity and protein content of PGC-1α were enhanced in skeletal muscle [7].

To regulate the deficiencies of myocyte activity in elderly subjects, many methods are applied such as physical training,

amino acid treatment, myostatin inhibition, testosterone treatment, calorie restriction, and noninvasive physical procedures like ultrasound therapy, electrical current modalities, and phototherapy [8]. The phototherapy of photobiomodulation (PBM) can effectively stimulate or inhibit biological functions, without irreducible damage [9]. It is well known that low level laser irradiation (LLLI) or monochromatic light has excellent therapeutic response in sarcopenia of ovariectomized rats [8] and cryoinjured rats [10]. However, the underlying molecular mechanisms are still unknown. We hypothesize that the LLLI may be effective in preventing sarcopenia in aged rats by reducing myocyte apoptosis, especially in type II fiber containing skeletal muscles, which are more susceptible to muscle mass losing via intrinsic apoptotic pathway. We further hypothesize that the age-related loss of muscle mass and myocyte apoptosis could be delayed by LLLI, an effect involving upregulation of insulin-like growth factor 1 (IGF-1) protein and SIRT1/PGC-1α axis messenger ribonucleic acid (mRNA) expression in skeletal muscle of aged rats. Therefore, this study was intended to investigate the alterations in BAX, Bcl-2, and IGF-1 protein expression under the irradiation of GaAlAs (810 nm; 125 mW/cm^2; 3.75 J/cm^2; 0.4 cm^2; 30 s). The effect of LLLI on SIRT1/PGC-1α axis mRNA expression in skeletal muscle of aged rats was also investigated.

2. Materials and Methods

2.1. Animals. Twenty female Sprague Dawley rats (18 months old, 378 ± 11 g) obtained from the experimental animal center in Guangzhou University of Traditional Chinese Medicine (GUTCM) (Guangzhou, China) were housed in plastic cages (five rats per cage) in a temperature controlled room (22 ± 2°C), with lights switched on from 6 am to 6 pm. All experimental procedures and animal care were approved by the Experimental Animal Care and Use Committee of GUTCM.

2.2. Low Level Laser Irradiation. The gallium-aluminum-arsenium (GaAlAs) laser (λ = 810 nm) was operated at 5 W, using a continuous wavelength of 810 nm with a 50 mW irradiation over each illuminated area of 0.4 cm^2. Phototherapy was started on day 1. The center of the greater trochanter was located by palpation. The intensity of 125 mW/cm^2 was performed punctually, through a single point contact mode in the center of the greater trochanter of the right femur [8] for 30 s every 24 h over an 8-week period.

2.3. Tissue Sampling and Preparation. On the last day of the experimental period, all rats were anaesthetized with pentobarbitone sodium (40 mg/kg) 24 h after LLLI irradiation. Bilateral gastrocnemius medialis muscles were then extracted and weighed. The proximal halves of the muscles were embedded in tragacanth gum, after which the samples were frozen in isopentane cooled by liquid nitrogen and were stored in a deep freeze (−80°C). And the distal halves of the muscles were immediately separated into the two blocks of superficial and deep regions, with each trimmed into 50 mg tissue samples. The superficial region of gastrocnemius muscle was organized with fast twitch fibres almost exclusively; however, the deep region included slow- and fast-twitch fibres [11]. The superficial region of gastrocnemius was homogenized in 0.01 M phosphate buffer (PBS; pH7.4). And then the homogenates were centrifuged at 4°C at 5600 g for 10 min; the supernatants were harvested and stored in a deep freeze (−80°C). The amount of protein in each muscle supernatant was determined by a BCA Protein Assay Kit (Pierce, Rockford, IL, USA).

2.4. Bax and Bcl-2 and IGF-1 Proteins Content. Proteins content for Bax, Bcl-2, and IGF-1 were determined by western immunoblot analysis (Figure 1). Separating gel (375 mM Tris-HCl; pH8.8; 0.4% SDS; 10% acrylamide) and stacking gel (125 mM Tris-HCl; pH6.8; 0.4% SDS; 10% acrylamide monomer) solutions were made, and polymerization was initiated by TEMED and ammonium persulfate. Then separating and stacking gels were quickly poured into a Bio-Rad Protein III gel-box (Bio-Rad; Hercules, CA, USA). Eighty micrograms of protein from skeletal muscle homogenates in sample buffer (Tris pH6.8 with 2% SDS, 30 mM DTT, 25% glycerol) were then loaded into the wells of 10% polyacrylamide gels and electrophoresed at 150 V. The gels were then transferred at 30 V onto a nitrocellulose membrane (Bio-Rad) overnight. The membranes were blocked in 5% nonfat milk in PBS with 0.1% Tween-20 at room temperature for 6 h. After blocking, the membranes were incubated at room temperature in PBS, with appropriate primary antibodies for 12 h: rabbit polyclonal Bax (1 : 200, Santa Cruz Biotechnology, CA, USA), mouse monoclonal Bcl-2 (1 : 250, BD Transduction Laboratories, KY, USA), and rabbit polyclonal IGF-1 (1 : 200, Santa Cruz Biotechnology). After three times of washing in PBS with 0.4% Tween-20, the membranes were incubated with horseradish peroxidase- (HRP-) conjugated secondary antibodies (Santa Cruz Biotechnology) in PBS at room temperature for 90 min. After three times of washing in PBS with 0.4% Tween-20, an enhanced chemiluminescence (ECL) detection system (Amersham: Piscataway, NJ, USA) was used for visualization. Densitometry (as area time's grayscale relative to background) was performed by using a Kodak film cartridge and film, a scanner interfaced with a microcomputer, and the NIH Image J Analysis program. The consistent loading of wells was confirmed with Ponceau-S-staining, and protein expression was quantified as area time's grayscale relative to background per mg protein.

2.5. SIRT1/PGC-1α Axis mRNA Content. Total RNA was prepared from 100 mg of frozen muscle tissues using TRIzol (Invitrogen, Singapore) and was purified according to the instructions included. The RNA purity was verified by the OD260/OD280 on the ultraviolet spectrophotometric module of the Tecan Microplate Reader (Infinite 200, Switzerland). Double-stranded cDNA was synthesized from −1 μg of total RNA using ReverTra Ace qPCR RT Kit (Osaka, Japan). Real-time PCR reactions were set up by using the SYBR-Green PCR kit (Osaka, Japan), and were cycled in StepOne Real-Time PCR System (Applied Biosystems, Foster City, CA, USA). The mRNA abundance of the targeted genes was

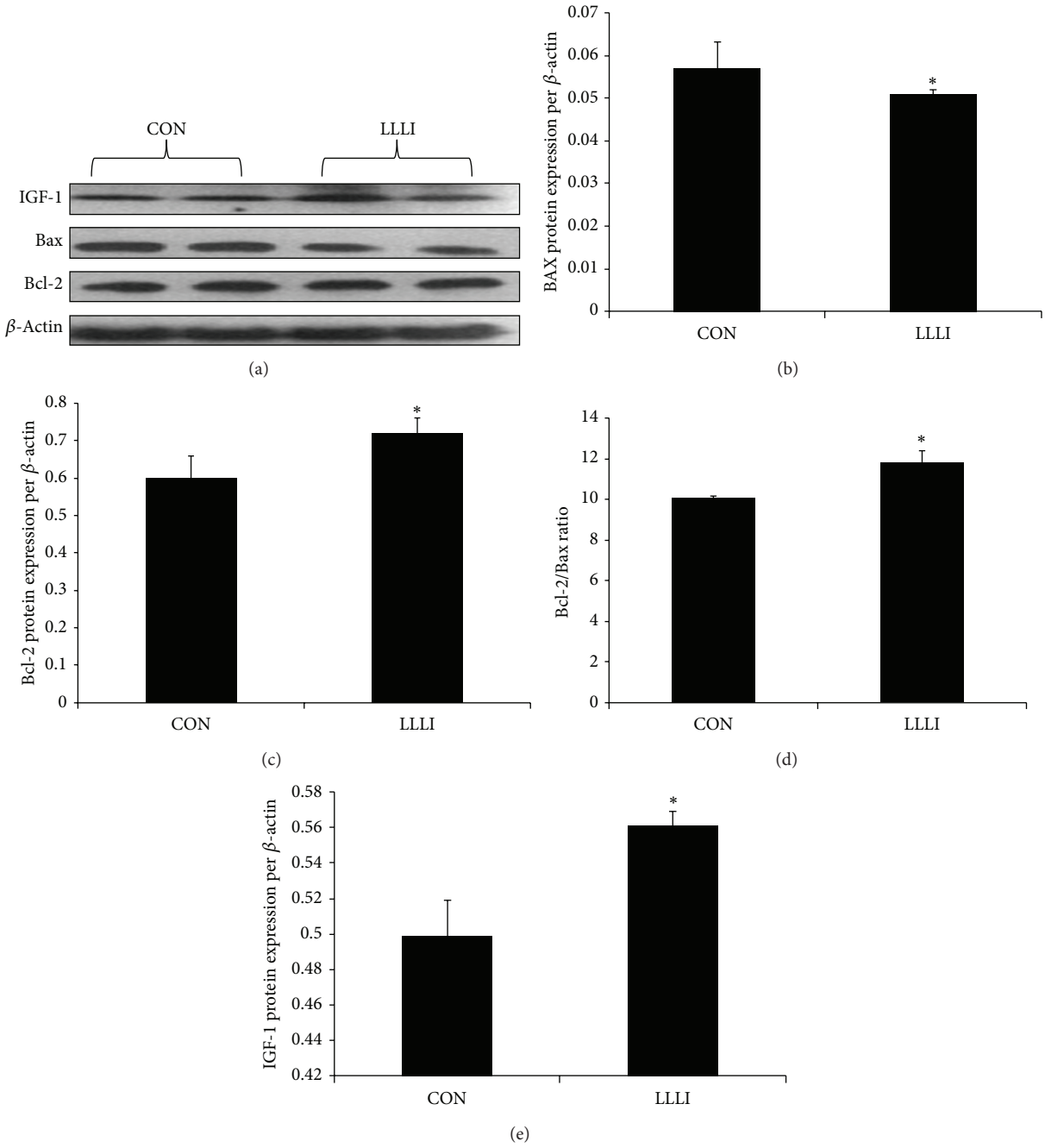

Figure 1: Western blot analysis and densitometry ratios of BAX, Bcl-2, and IGF-1 in gastrocnemius. Western blot analysis is used to determine the level of proteins extracted from gastrocnemius, relative to the endogenous control β-actin. Western blots and values are means ± SE representative from one rat from each group. $n = 10$ for each group. $^{*}P < 0.05$ versus control group.

normalized to β-actin. Primer pairs were designed based on GenBank reference sequences and were listed in Table 1.

2.6. Statistical Analysis. All data were presented as mean ± SE. All differences were analyzed by the student's t-test. And, in all analyses, $P < 0.05$ was considered statistically significant.

3. Results

3.1. Gastrocnemius Wet Weight and Gastrocnemius Index. Table 2 summarizes the effects of 8-week LLLI on body mass and gastrocnemius muscle weights. The gastrocnemius muscle weights of the LLLI group are significantly increased compared to the CON group ($P < 0.05$), although the animal body mass was not changed ($P > 0.05$). There is a significant

Table 1: Primers used for PCR amplification of cDNA.

Genes	Sense	Antisense
SIRT1	CTGTTTCCTGTGGGATACCTGACT	ATCGAACATGGCTTGAGGATCT
PGC-1a	CTACAATGAATGCAGCGGTCTT	TGCTCCATGAATTCTCGGTCTT
NGF1	GCATTGAGCTACTGACAGAC	CTGTGTCCTGGATCTTCCTT
TFAM	TGGAACACAGCCACATGCTT	ACCATGGTGGCAAACTGTCT
GAPDH	CTATCGGCAATGAGCGGTT	TGTGTTGGCATAGAGGTCTTTA
SOD2	CGTCATTCACTTCGAGCAGA	AAAATGAGGTCCTGCAGTGG
Caspase 3	TGGTTCATCCAGTCGCTTTGT	CAAATTCTGTTGCCACCTTTCG

Table 2: Weight, gastrocnemius weight, and gastrocnemius mass/body mass.

Groups	Body mass (g)	Gastrocnemius weight (g)	Gastrocnemius mass/body mass
CON	378.8 ± 65.7	0.61 ± 0.16	1.60 ± 0.20
LLLI	378.0 ± 27.6	0.84 ± 0.03*	2.20 ± 0.11*

* $P < 0.05$ versus the CON groups.

increase in the gastrocnemius mass/body mass in the LLLI group compared to the CON group ($P < 0.05$).

3.2. Protein Level of Bax, Bcl-2, IGF-1, and Bcl-2/BAX Ratio. Bcl-2 and IGF-1 protein expression in the gastrocnemius of the LLLI group are significantly elevated, while BAX expression is significantly inhibited, compared to the CON group ($P < 0.05$). Therefore, LLLI significantly increases Bcl-2/BAX ratio compared to CON group ($P < 0.05$).

3.3. Expression of SIRT1/PGC-1α Axis Genes mRNA. The mRNA contents of SIRT1, PGC-1α, TFAM, NRF1, and SOD2 in the gastrocnemius of LLLI group are significantly upregulated compared to the CON group ($P < 0.05$). However, caspase 3 mRNA content in the gastrocnemius of the LLLI group significantly decreased compared to CON group ($P < 0.05$).

4. Discussions

4.1. LLLI Effects on Age-Related Loss of Muscle Mass in Aged Rats. It has been confirmed in many studies that 30% or more of skeletal muscle myocytes, particularly in fast-twitch fibers, may be lost with aging due to apoptosis. Andersen et al. [12]. found gastrocnemius mass/body mass ratios significantly decreased in aged rats (22 months) by 22.8% compared to young rats (6 months). Gastrocnemius muscle weight and gastrocnemius muscle wt/body were 24% and 20% lower in aged rats (22 months) compared to young rats (3 months), respectively [13]. Song et al. [14] found that gastrocnemius mass decreased by 11.2% in aged rats (22 months) compared to rats of 18 months.

It has been suggested that the age-related loss of muscle mass may be inhibited by LLLI [9]. Corazza et al. [8] demonstrated that LLLI improved the muscle volume of middle aged ovariectomized rats. In present study, the gastrocnemius muscle weight and gastrocnemius mass/body mass ratios of the LLLI group are 30% and 37.5% higher than the CON group, respectively. In other words, PBM from LLLI retarded the loss of skeletal muscle mass from aged rats (18 months) and has larger muscle mass in old rats (22 months) compared to younger ones (6 months).

4.2. LLLI Effects on Apoptotic Signal in Aging Skeletal Muscle. A large number of studies using both rodent model and human subjects have confirmed that the mechanism for age-associated skeletal muscle fiber atrophy and myocyte loss is due to apoptosis [2, 3]. A critical event in mitochondrial-driven apoptosis is the formation of permeable membrane pores, regulated by the balance between competing antiapoptotic Bcl-2 family proteins such as Bcl-2 and proapoptotic proteins including Bax. Increased proapoptotic signaling through the mitochondrial Bcl-2 family has been implicated as an important mechanism leading towards muscle cell loss and atrophy with aging [2]. Previously, elevation of Bax and caspase 3 protein expression and Bax/Bcl-2 ratio in aging skeletal muscle was reported by Alway et al. [15]. They showed that the dysregulation of Bcl-2 pathway signaling leads to caspase 3 activation and intrinsic apoptotic pathways in age-associated skeletal muscle [15]. And Song et al. found that the mitochondrial Bcl-2 protein levels were reduced by 20% in aged rats compared to young rats, but the mitochondrial Bax protein level was 11% higher in aged rats compared to young rats. Moreover, the Bcl-2/Bax ratio in white gastrocnemius was increased by 98% with age [14]. In addition, caspase 3 mRNA content in aged rats (22 months) was 72% higher than that in young rats (4 months) [16].

Theoretically, the apoptotic signal in aging skeletal muscle may be partly wiped out by the LLLI [9]. Data from aged rats indicate that LLLI can provide protection against sarcopenia [8, 10]. Studies on experimental animal and various cell types revealed that LLLI acted as a preventive tool against cell apoptosis [17, 18]. And other studies indicated that LLLI may inhibit muscle cell and myoblasts apoptosis through upstream Bcl-2 family signaling [19, 20]. LLLI inhibited apoptosis of muscle cell after irradiation with fluency of 133.3 J/cm^2 in the rats gastrocnemius before high intensity exercise [19]. In the present study, LLLI resulted in a reduction of Bax protein and elevation of Bcl-2 protein expression and

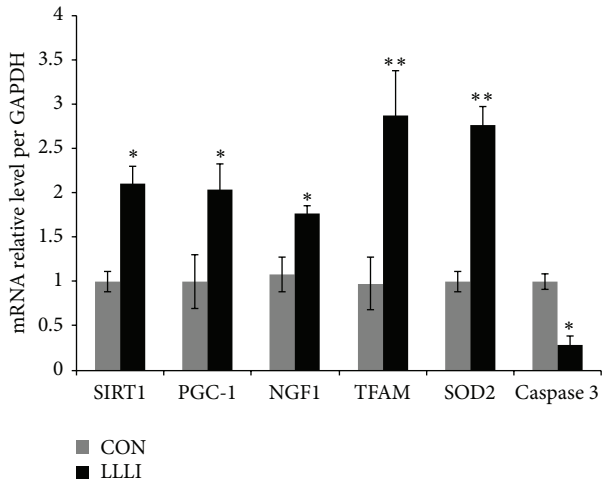

Figure 2: mRNA quantification of SIRT1/PGC-1α axis genes in skeletal muscle subjected to 8-week LLLI. Real-time PCR is used to determine the expression of genes in gastrocnemius from the LLLI group or the CON group, relative to the endogenous control GAPDH. Values are means ± SE. $n = 10$ for each group. $^{*}P < 0.05$, $^{**}P < 0.01$ versus CON group.

the Bcl-2/Bax ratio in the white gastrocnemius of old rats (Figure 2). Remarkably, LLLI diminished caspase 3 mRNA level by over 95%, indicating a robust effect on key integrative regulator of apoptosis. Our results suggested the contributing mechanism by which the LLLI prevents muscle wasting with age and elevates Bax/Bcl-2 ratio by decreasing Bax protein and increasing Bcl-2 protein expression.

4.3. LLLI Effects on IGF-I Protein Expression in Aging Skeletal Muscle. IGF-1 may be a major factor in muscle regrowth during recovery from muscle injury. IGF-1 may control skeletal muscle growth in terms of both satellite cell proliferation [21] and myofiber hypertrophy secondary to changes in protein synthesis [22]. Further, IGF-1 might also be cytoprotective in maintaining cell survival signaling by ameliorating apoptotic signaling during oxidative stress [23]. Data from aging rats also indicated that an age-related impairment of IGF-1 expression and cell survival signaling potentially may play a role in the apoptosis of skeletal muscle with age [23].

IGF-I protein expression in aging skeletal muscle may theoretically be promoted with LLLI [9]. Luo et al. [24] reported that LLLI at 635 nm increased the expression of IGF-1 1, 2, and 3 days after injury. Corazza et al. [8] demonstrated that LLLI at 850 nm could reverse ovariectomized-induced downregulation of IGF-1 protein expression in skeletal muscle. Enhanced IGF-1 production in response to LLLI inhibited atrophy muscle volume in middle-age ovariectomized rats by attenuating the production of TNF-α and reactive oxygen species [8], which induced muscle apoptosis and sarcopenia [25]. In the present study, our data showed that IGF-1 protein expression in aged rat was significantly promoted by the LLLI at 810 nm. Taken together, these results directly suggested that LLLI inhibited skeletal muscle apoptosis, probably through stimulating the IGF-1 production, thereby improving the muscle mass in aged rats.

4.4. LLLI Effects on SIRT1-PGC-1α Pathway mRNA Expression in Aging Skeletal Muscle. Age-related loss of SIRT1 mRNA may contribute to the decline in mitochondrial function with aging. Alterations in mitochondrial function and low mitochondriogenesis were considered major factors underlying sarcopenia [26]. Damaged mitochondria may be not only less bioenergetically efficient but also more ready for apoptosis. SIRT1, a NAD^{+}-dependent histone deacetylase [5], may be implicated in the prevention of many age-related diseases such as sarcopenia, cancer, Alzheimer's disease, and type 2 diabetes by maintaining mitochondrial homeostasis [6]. In contrast, skeletal muscle aging may be associated with a downregulation of the SIRT1-mediated transcriptional pathway of mitochondrial biogenesis that impinge on multiple aspects of mitochondrial homeostasis [27]. The SIRT1 protein was found not to be affected by age in the white gastrocnemius in one study [16], but the SIRT1 mRNA was found to be significantly lower by 54.2% in aged rats (22 months) compared to young rats (4 months) in the other study [28].

SIRT1 mRNA expression in aging skeletal muscle may be theoretically promoted with LLLI [9]. In the present study, we found the SIRT1 mRNA content treated by LLLI increased 2.1-fold in the white gastrocnemius of aged rats (Figure 2). Moreover, the study on satellite cells [29] and differentiated muscle cells [30] indicated that LLLI promoted SIRT1 deacetylase activity, probably through increased NAD^{+} level or the enhancement of SIRT1 protein expression.

The mechanism of LLLI promotion of SIRT1 expression and NAD^{+} level has not been well elucidated. LLLI was found to induce a significant increase in cAMP level via upregulating ATP content and mitochondrial membrane potential [31]. As a second messenger, cAMP may initiate beneficial cell signaling pathways leading to the activation of redox sensitive transcription factors such as nicotinamide phosphoribosyltransferase (NAMPT) and protein kinase A (PKA) signaling pathways, which may increase NAD^{+} level and induce expression of IGF-1, respectively [32]. IGF-I may enhance muscle cell survival signaling in terms of protection from oxidative stress-induced mitochondrial-driven apoptosis through upregulating the SIRT1 expression [33]. We then proposed that the downregulation of the cAMP-NAMPT-NAD^{+} signaling pathway may contribute to mitochondrial deterioration in aged muscle, whereas LLLI may ameliorate the deficits. In addition, the overexpression of anticancer genes cyclin-dependent kinase inhibitor,$p16^{INK4a}$, in satellite cells irreversibly may affect their intrinsic regenerative and self-renewal capacities in sarcopenic muscles. Recent studies showed that cAMP-PKA signaling was shown to inhibit the expression of $p16^{INK4a}$ [34], which appeared to be positively associated with reduced myogenic potential and increased cellular senescence in satellite cells from physiologically aged individuals with sarcopenia through downregulation of the SIRT1 expression [35, 36]. We further proposed that LLLI may promote the muscle-regeneration process by regulating PKA-$p16^{INK4a}$-SIRT1 pathway in sarcopenic muscles (Figure 3).

SIRT1 has been found to increase the transcription of PGC-1α and activate PGC-1α by deacetylation [37, 38].

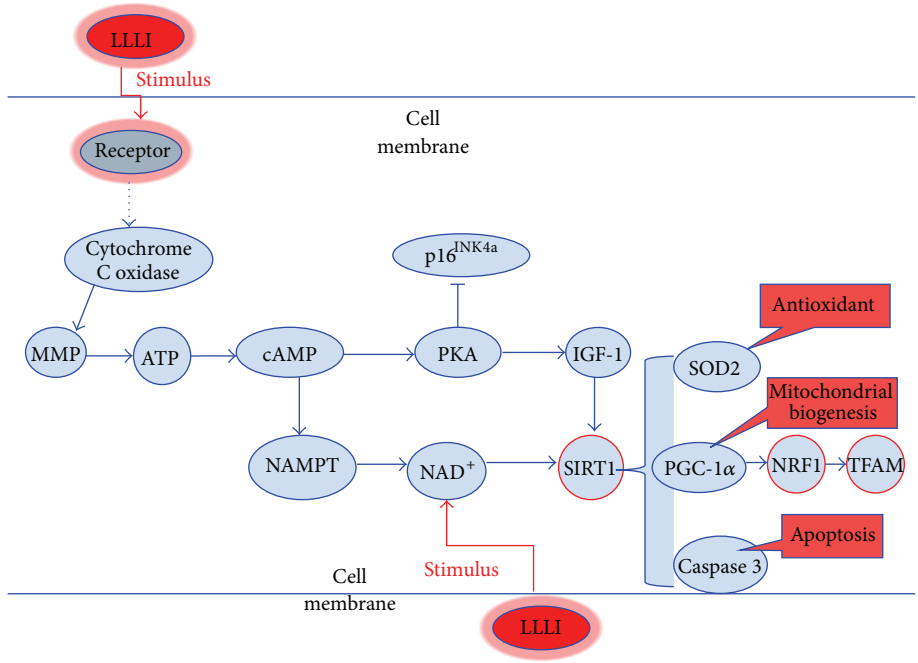

Figure 3: Schematic depiction of the cellular signaling pathways triggered by LLLI. After photons may be absorbed by chromophores in the cell membrane, mitochondria cytochrome C oxidase, MMP, ATP, and cAMP may be increased, but signaling pathways such as PKA/IGF-1 may be also increased, contributing to increased NAMPT/SIRT1 pathway and inhibited expression of $p16^{INK4a}$.

The mRNA expression of PGC-1α is significantly decreased by 35% in skeletal muscles of old rats (22 months) compared to young rats (4 months) as previously described [16]. As expected, the mRNA expression of PGC-1α and SIRT1 was found to be significantly correlated [28]. Interestingly, the expression of mitochondrial biogenesis genes was known to be under the control of the PGC-1α and their activator SIRT1. And increased PGC-1α activity may enhance its DNA binding and thus induce the expression of genes involved in mitochondrial biogenesis. And nuclear respiratory factors 1 (NRF1) may promote the expression of most nuclear-encoding mitochondrial proteins, as well as mitochondrial transcription factor A (TFAM) that may directly stimulate mitochondrial DNA replication and transcription. Thus, our study suggested that the LLLI may increase the transcription of NRF1 and TFAM, probably through upregulating the transcription of SIRT1 and PGC-1α. Nguyen et al. [30] confirmed that SIRT1 and PGC-1α expression itself was elevated in C2C12 cells following 4 days of LLLI. Their results showed that modulation of mitochondrial regulation via LLLI represented one of the molecular and cellular mechanisms contributing to the clinical therapeutic benefits of LLLI in patients with musculoskeletal injuries [30]. Furthermore, our data indicated that 8 weeks of LLLI were useful for the treatment of other skeletal muscle disorders with dysfunctional mitochondria contributing towards their etiology in sarcopenic muscles probably through modulation of SIRT1/PGC-1α axis and its key regulators. However, further studies are needed to verify the mechanism of action.

The skeletal muscle of aged rats may be characterized by enhanced reactive oxygen species production compared with young rats. Aging may be associated with increased oxidative damage, leading to muscle dysfunction [39]. Aged animals displayed a significant decrease by 59.3% in the transcript levels of mitochondrial antioxidative enzyme SOD2, compared to the younger ones [28]. In the present study, we found that SOD2 mRNA level increased 2.8-fold by the LLLI (Figure 2). Thus, age-related loss of SOD2 mRNA may contribute to the oxidative damage with aging, which could be inhibited by LLLI. Since SIRT1 has been found to increase the transcription of SOD2 by activating PGC-1α [40], we proposed that LLLI may promote SIRT1-mediated gene transcription of SOD2.

We recognize that our study evaluated skeletal muscle mass, marker of apoptosis, and gene expression of the expression of a group of genes involved in mitochondrial function and biogenesis in an animal model, so we understand that this represents a limitation and we express caution at extrapolating our findings into humans at this stage. Nevertheless, LLLI has a strong safety profile and reports of side effects in an evidence base of over 200 randomized controlled clinical trials are few and minor. Therefore, we believe serious consideration should be given to the potential of LLLI as a treatment option of long-term conditions like sarcopenia. Future studies would include investigation of the effects of LLLI on protein expression of mitochondrial biogenesis and oxidative capacity and functional aspects of sarcopenia and the determination of optimal parameters to inform the design of robust clinical trials. We hope that our findings may initiate interest in the use of LLLI as a potentially useful adjunct for sarcopenia.

5. Conclusion

LLLI at 810 nm inhibits sarcopenia associated with upregulation of Bcl-2/BAX ratio and IGF-1 and SIRT1/PGC-1α axis mRNA expression in aged rats. This suggests that LLLI may inhibit sarcopenia in the aged rat by promoting the expression of IGF-1 and SIRT1/PGC-1α axis genes. Further studies are needed to verify the mechanism of action and effects on functional outcomes and to establish optimal parameters of application to inform clinical use.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

Authors' Contribution

Fang-Hui Li and Yan-Ying Liu contributed equally to this study and share first authorship.

Acknowledgments

This work was supported by National Science Foundation of China (60878061) and Guangdong Scientific Project (2012B031600004).

References

[1] W. K. Mitchell, J. Williams, P. Atherton, M. Larvin, J. Lund, and M. Narici, "Sarcopenia, dynapenia, and the impact of advancing age on human skeletal muscle size and strength; a quantitative review," *Frontiers in Physiology*, vol. 3, article 260, 2012.

[2] A. Dirks and C. Leeuwenburgh, "Apoptosis in skeletal muscle with aging," *American Journal of Physiology: Regulatory Integrative and Comparative Physiology*, vol. 282, no. 2, pp. R519–R527, 2002.

[3] E. Marzetti, H. A. Lees, T. M. Manini et al., "Skeletal muscle apoptotic signaling predicts thigh muscle volume and gait speed in community-dwelling older persons: an exploratory study," *PLoS ONE*, vol. 7, no. 2, Article ID e32829, 2012.

[4] R. Calvani, A.-M. Joseph, P. J. Adhihetty et al., "Mitochondrial pathways in sarcopenia of aging and disuse muscle atrophy," *Biological Chemistry*, vol. 394, no. 3, pp. 393–414, 2013.

[5] T. Finkel, C.-X. Deng, and R. Mostoslavsky, "Recent progress in the biology and physiology of sirtuins," *Nature*, vol. 460, no. 7255, pp. 587–591, 2009.

[6] S. D. Westerheide, J. Anckar, S. M. Stevens Jr., L. Sistonen, and R. I. Morimoto, "Stress-inducible regulation of heat shock factor 1 by the deacetylase SIRT," *Science*, vol. 323, no. 5917, pp. 1063–1066, 2009.

[7] T. Wenz, S. G. Rossi, R. L. Rotundo, B. M. Spiegelman, and C. T. Moraes, "Increased muscle PGC-1α expression protects from sarcopenia and metabolic disease during aging," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 106, no. 48, pp. 20405–20410, 2009.

[8] A. V. Corazza, F. R. Paolillo, F. C. Groppo, V. S. Bagnato, and P. H. F. Caria, "Phototherapy and resistance training prevent sarcopenia in ovariectomized rats," *Lasers in Medical Science*, vol. 28, no. 6, pp. 1467–1474, 2013.

[9] T. C.-Y. Liu, Y. - Liu, E.-X. Wei, and F.-H. Li, "Photobiomodulation on stress," *International Journal of Photoenergy*, vol. 2012, Article ID 628649, 11 pages, 2012.

[10] F. Vatansever, N. C. Rodrigues, L. L. Assis et al., "Low intensity laser therapy accelerates muscle regeneration in aged rats," *Photonics & Lasers in Medicine*, vol. 1, no. 4, pp. 287–297, 2012.

[11] P. E. Mozdziak, P. M. Pulvermacher, and E. Schultz, "Muscle regeneration during hindlimb unloading results in a reduction in muscle size after reloading," *Journal of Applied Physiology*, vol. 91, no. 1, pp. 183–190, 2001.

[12] N. B. Andersen, T. T. Andreassen, H. Orskov, and H. Oxlund, "Growth hormone and mild exercise in combination increases markedly muscle mass and tetanic tension in old rats," *European Journal of Endocrinology*, vol. 143, no. 3, pp. 409–418, 2000.

[13] S. A. Spier, M. D. Delp, J. N. Stallone, J. M. Dominguez II, and J. M. Muller-Delp, "Exercise training enhances flow-induced vasodilation in skeletal muscle resistance arteries of aged rats: role of PGI2 and nitric oxide," *American Journal of Physiology: Heart and Circulatory Physiology*, vol. 292, no. 6, pp. H3119–H3127, 2007.

[14] W. Song, K. J. Ko, S. J. Shin, and D. S. Ryu, "Penile abscess secondary to neglected penile fracture after intracavernosal vasoactive drug injection," *The World Journal of Men's Health*, vol. 30, no. 3, pp. 189–191, 2012.

[15] S. E. Alway, H. Degens, G. Krishnamurthy, and C. A. Smith, "Potential role for Id myogenic repressors in apoptosis and attenuation of hypertrophy in muscles of aged rats," *American Journal of Physiology: Cell Physiology*, vol. 283, no. 1, pp. C66–C76, 2002.

[16] C. Kang, E. Chung, G. Diffee, and L. L. Ji, "Exercise training attenuates aging-associated mitochondrial dysfunction in rat skeletal muscle: role of PGC-1α," *Experimental Gerontology*, vol. 48, no. 11, pp. 1343–1350, 2013.

[17] M. Wu and T. C.-Y. Liu, "Single-cell analysis of protein kinase C activation during anti-apoptosis and apoptosis induced by laser irradiation," *Photomedicine and Laser Surgery*, vol. 25, no. 2, pp. 129–130, 2007.

[18] K. K. Yip, S. C. L. Lo, M. C. P. Leung, K. F. So, C. Y. Tang, and D. M. Y. Poon, "The effect of low-energy laser irradiation on apoptotic factors following experimentally induced transient cerebral ischemia," *Neuroscience*, vol. 190, pp. 301–306, 2011.

[19] D. A. Sussai, P. D. T. C. D. Carvalho, D. M. Dourado, A. C. G. Belchior, F. A. Dos Reis, and D. M. Pereira, "Low-level laser therapy attenuates creatine kinase levels and apoptosis during forced swimming in rats," *Lasers in Medical Science*, vol. 25, no. 1, pp. 115–120, 2010.

[20] G. Shefer, T. A. Partridge, L. Heslop, J. G. Gross, U. Oron, and O. Halevy, "Low-energy laser irradiation promotes the survival and cell cycle entry of skeletal muscle satellite cells," *Journal of Cell Science*, vol. 115, part 7, pp. 1461–1469, 2002.

[21] H. Schmalbruch and U. Hellhammer, "The number of nuclei in adult rat muscles with special reference to satellite cells," *The Anatomical Record*, vol. 189, no. 2, pp. 169–175, 1977.

[22] T. H. Bark, M. A. McNurlan, C. H. Lang, and P. J. Garlick, "Increased protein synthesis after acute IGF-I or insulin infusion is localized to muscle in mice," *American Journal of Physiology: Endocrinology and Metabolism*, vol. 275, no. 1, part 1, pp. E118–E123, 1998.

[23] S. Y. Yang, M. Hoy, B. Fuller, K. M. Sales, A. M. Seifalian, and M. C. Winslet, "Pretreatment with insulin-like growth factor i protects skeletal muscle cells against oxidative damage via

PI3K/Akt and ERK1/2 MAPK pathways," *Laboratory Investigation*, vol. 90, no. 3, pp. 391–401, 2010.

[24] L. Luo, Z. Sun, L. Zhang, X. Li, Y. Dong, and T. C.-Y. Liu, "Effects of low-level laser therapy on ROS homeostasis and expression of IGF-1 and TGF-β1 in skeletal muscle during the repair process," *Lasers in Medical Science*, vol. 28, no. 3, pp. 725–734, 2013.

[25] T. Philips and C. Leeuwenburgh, "Muscle fiber specific apoptosis and TNF-α signaling in sarcopenia are attenuated by lifelong calorie restriction," *FASEB Journal*, vol. 19, no. 6, pp. 668–670, 2005.

[26] F. Derbre, B. Ferrando, M. C. Gomez-Cabrera et al., "Inhibition of xanthine oxidase by allopurinol prevents skeletal muscle atrophy: role of p38 MAPKinase and E3 ubiquitin ligases," *PLoS ONE*, vol. 7, no. 10, Article ID e46668, 2012.

[27] A. P. Gomes, N. L. Price, and A. J. Ling, "Declining NAD^+ induces a pseudohypoxic state disrupting nuclear-mitochondrial communication during aging," *Cell*, vol. 155, no. 7, pp. 1624–1638, 2013.

[28] A.-L. Charles, A. Meyer, S. Dal-Ros et al., "Polyphenols prevent ageing-related impairment in skeletal muscle mitochondrial function through decreased reactive oxygen species production," *Experimental Physiology*, vol. 98, no. 2, pp. 536–545, 2013.

[29] F. H. Li, E. X. Wei, Y. Y. Liu, and T. C. Y. Liu, "Redundant photobiomodulation on low glucose induced dysfunctions of C2C12 myoblasts," *Lasers in Surgery and Medicine*, vol. 44, supplement 24, p. 63, 2012.

[30] L. M. Nguyen, A. G. Malamo, K. A. Larkin-Kaiser, P. A. Borsa, and P. J. Adhihetty, "Effect of near-infrared light exposure on mitochondrial signaling in C2C12 muscle cells," *Mitochondrion*, vol. 14, no. 1, pp. 42–48, 2014.

[31] W.-P. Hu, J.-J. Wang, C.-L. Yu, C.-C. E. Lan, G.-S. Chen, and H.-S. Yu, "Helium-neon laser irradiation stimulates cell proliferation through photostimulatory effects in mitochondria," *Journal of Investigative Dermatology*, vol. 127, no. 8, pp. 2048–2057, 2007.

[32] S.-J. Park, F. Ahmad, A. Philp et al., "Resveratrol ameliorates aging-related metabolic phenotypes by inhibiting cAMP phosphodiesterases," *Cell*, vol. 148, no. 3, pp. 421–433, 2012.

[33] I. V. Kravchenko, V. A. Furalyov, and V. O. Popov, "Stimulation of mechano-growth factor expression by myofibrillar proteins in murine myoblasts and myotubes," *Molecular and Cellular Biochemistry*, vol. 363, no. 1-2, pp. 347–355, 2012.

[34] J. Liu, H. Liu, and Y. Liang, "Effect of compound Chinese drug bailong on the expression of tumor suppressor genes and relationship with prekallikrein activator signal pathway in human gastric carcinoma BGC82-3 cell line," *Zhongguo Zhong Xi Yi Jie He Za Zhi*, vol. 19, no. 10, pp. 613–616, 1999.

[35] P. Sousa-Victor, S. Gutarra, L. García-Prat et al., "Geriatric muscle stem cells switch reversible quiescence into senescence," *Nature*, vol. 506, no. 7488, pp. 316–321, 2014.

[36] Y. Li and T. O. Tollefsbol, "P16INK4A suppression by glucose restriction contributes to human cellular lifespan extension through SIRT1-mediated epigenetic and genetic mechanisms," *PLoS ONE*, vol. 6, no. 2, Article ID e17421, 2011.

[37] S. Nemoto, M. M. Fergusson, and T. Finkel, "SIRT1 functionally interacts with the metabolic regulator and transcriptional coactivator PGC-1α," *The Journal of Biological Chemistry*, vol. 280, no. 16, pp. 16456–16460, 2005.

[38] B. J. Wilson, A. M. Tremblay, G. Deblois, G. Sylvain-Drolet, and V. Giguère, "An acetylation switch modulates the transcriptional activity of estrogen-related receptor α," *Molecular Endocrinology*, vol. 24, no. 7, pp. 1349–1358, 2010.

[39] P. A. Figueiredo, S. K. Powers, R. M. Ferreira, H. J. Appell, and J. A. Duarte, "Aging impairs skeletal muscle mitochondrial bioenergetic function," *Journals of Gerontology A: Biological Sciences and Medical Sciences*, vol. 64, no. 1, pp. 21–33, 2009.

[40] Y. Olmos, I. Valle, S. Borniquel et al., "Mutual dependence of Foxo3a and PGC-1α in the induction of oxidative stress genes," *The Journal of Biological Chemistry*, vol. 284, no. 21, pp. 14476–14484, 2009.