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SIRT1-related inhibition of pro-inflammatory responses and oxidative stress are involved in the mechanism of nonspecific low back pain relief after exercise through modulation of Toll-like receptor 4

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Low back pain is a common clinical problem that causes disability and impaired quality of life. While the reason behind low back pain was largely considered to be of musculoskeletal origin, the contribution of inflammatory cytokines and oxidative stress could never be overlooked. Exercise has been proven to be an effective approach to treat low back pain. However, the mechanism of the exercise effect on the inflammatory cytokines and oxidative stress is still largely unknown. In this study, we revealed that exercise intervention reduces Toll-like receptor 4 (TLR-4) pathway and enhances Sirtuin 1 (SIRT1) expression in low back pain patients. We also confirmed that exercise up-regulates the expression of peroxisome proliferator-activated receptor-gamma, PPAR-γ coactivator-1 and FoxOs family proteins and also increases the activity of catalase and superoxide dismutase in patients with low back pain. Furthermore, we found that exercise intervention attenuates the oxidative stress, pro-inflammatory cytokine concentrations and p53 expression in patients with low back pain. This study demonstrates that exercise intervention improves low back pain symptoms through regulation of the SIRT1 axis with repression of oxidative stress and TLR-4 inhibition.

Keywords: exercise/genes/low back pain/SIRT1/toll like receptor 4.

Abbreviations: IFN-γ, interferon-γ; IP-10, IFN-γ-induced protein 10; NF-κB, nuclear factor-kappa B; PGC-1a, PPAR-γ coactivator-1 alpha; PPAR-γ, peroxisome proliferator-activated receptor-gamma; ROS, reactive oxygen species; SIRT1, Sirtuin 1; SOD, superoxide dismutase; TLR-4, Toll-like receptor 4.

Low back pain is a common clinical symptom, and is defined as pain located in the posterior aspect of body from the lower margin of 12th rib to the lower gluteal folds with or without referring to unilateral or bilateral lower limbs. Approximately 80% of patients suffer from low back pain at some point in their lives (*1*). A recent systemic review article revealed low back pain ranked highest in terms of years lived with disability, and the global point prevalence of it was 9.4% (*2*). Although the prevalence and incidence is high, the precise source of low back pain cannot be identified in most cases, which is termed nonspecific mechanical low back pain (*3*). As a previous study suggested that 98% of low back pain disorders are caused by musculoskeletal problems (*4*), anatomic abnormalities cannot completely explain the origin of low back pain. A past study evidenced that cytokine network plays a significant role in the pathogenesis of low back pain (*5*). Igarashi *et al.* (*6*) suggested that there are higher levels of pro-inflammatory cytokines, such as IL-1β, TNF-α and IL-6, in patients with low back pain. In another study, high serum IL-6 levels, but not disc degeneration, were shown to be associated with less favourable recovery in patients with lumbar radicular pain (*7*). From the studies above, we can postulate that both inflammatory cytokines as well as musculoskeletal causes contribute significantly to the occurrence of low back pain.

Exercise has been shown to be one of the most effective approaches for the treatment of patients with low back pain. Exercise not only enhances spine stability but also provides functional improvement in patients with low back pain (*8*). Another systemic review article also showed pilates exercise offers greater improvements in pain and functional ability compared with usual care in patients with chronic low back pain (*9*). In addition to clinical improvement in pain and function, exercise can also modulate cytokine network *in vivo*. In past studies, exercise training has been shown to enhance expressions of neutrophic factors in the hippocampus of lipopolysaccharide-injected rats, represses neuronal cell death and inflammation represses and reduces pro-inflammatory responses in older people (*10–12*). Because the inflammatory cytokine plays a vital role in the pathogenesis of low back pain, we postulate that exercise may relieve low back pain, at least in part, by reducing the pro-inflammatory response inside our body.

Toll-like receptors (TLRs) are known as transmembrane molecules and microbe-sensing receptors with an important function in innate immunity. Intracellular

signalling pathways activated by TLRs promote pro-inflammatory events by mediating pro-inflammatory chemokines and cytokines (*13*). Increased expression of TLR-4 has been identified in diseases including rheumatoid arthritis and osteoarthritis (*14*). Activation of NF-kB is one of the main signalling pathway of TLR-4 which up-regulate pro-inflammatory cytokine genes causing inflammatory events (*15*, *16*). Another inflammation-related gene is Sirtuin 1 (SIRT1). SIRT1 is shown to be a nuclear metabolic sensor that regulate gene expression through deacetylation of histones, transcription factors and transcription co-factors. It plays a vital role in metabolism, development, reproduction, and can affect complex biological phenomena such as aging and disease (*17*). In mammals, physiological inflammation is regulated by SIRT1 through modulation of mitogen-activated protein kinase family phosphorylation. SIRT1 also plays a vital role in regulating inflammatory events through nuclear factor-kappa B (NF-κB) has two subunits, including p50 and p65, and SIRT1 can directly interact with p65 and regulate NF-κB transcriptional activity (*18*). Inhibition of SIRT1 expression has been shown to pu-regulates NF-κB activity and pro-inflammatory responses (*19*, *20*). Wuertz *et al.* (*21*) first reported that NF-κB activity is promoted and SIRT1 is mitigated in patients with nucleus pulposus related pain, and they concluded that decreased pro-inflammatory cytokines may possibly be the underlying mechanism of pain reduction observed *in vivo*. On the other hand, oxidative stress can inhibit SIRT1 activity, and decreased activity of SIRT1 enhances the NF-κB signalling, which induces inflammatory responses (*22*). Therefore, we postulated that exercise may reduce the inflammatory responses by enhancing SIRT1 activities and extenuating oxidative stress *in vivo*.

In summary, we hypothesized that exercise intervention can inhibit TLR-4 signalling, up-regulate SIRT1 and its downstream pathways, thereby reducing oxidative stress and pro-inflammatory responses. Therefore, we aimed to test whether TLR-4 is involved in non-specific low back pain after exercise intervention. We also tested if the oxidative stress, pro-inflammatory cytokines and SIRT1 are involved in low back pain after exercise treatment.

Materials and Methods

Patients

This study was approved by Taipei Veterans General Hospital Institutional Review Board (IRB:2013-08-007A, from August 30, 2013 to August 29, 2014), and all participants signed informed consents before recruiting into our study. Thirty patients (female:male = 15:15) with chief complaint of low back pain were recruited from the Department of Physical Medicine & Rehabilitation, Taipei Veterans General Hospital. The exclusion criteria are red flags of low back pain including those with history of lumbar trauma, malignancy, diabetes mellitus, immunosuppression, prolonged corticosteroid use, comorbid infection, focal neurological deficit, unexplained weight less and unexplained fever (*3*). Those with significant pathological changes visualized on plain film of lumbar spine were also excluded. Visual analogue scales were recorded during their initial hospital visits. Five microlitres venous blood samples were drawn from each patient before they started exercise training.

Exercise protocol

Each patient received 5 min of muscle stretch exercises were implemented in six directions of low back posture, and each posture were maintained for 30 s to improve flexibility. The six directions of low back posture were flexion, extension, side bending to right and left and rotation to right and left (*23*). Then, the patients received 10 min of back muscle strengthening exercises and 5 min of lower limb strengthening exercises. We selected the strengthening programme from Hicks's study (*24*, *25*), which included strengthening major stabilizers of the spine (*e.g.* oblique abdominis, multifidus, quadratus lumborum and erector spinae muscles) and lower limb muscles (*e.g.* quadriceps, hamstrings, gluteus maximus and gluteus medius). Each participants received physical therapy programmes mentioned above three times a week. Four weeks later, visual analogue scales of pain were recorded again, and another 5 ml of venous blood samples were drawn from each participants.

Isolation of mRNA and quantitative real-time polymerase chain reaction

Human blood was collected in BD K2-EDTA tubes (5 ml) from patients with low back pain before and after exercise intervention. Total blood RNA was isolated with the RNeasy Plus mini kit (Qiagen, Hilden, Germany). The quality of RNA was confirmed via an Experion Automated Electrophoresis Station (Bio-Rad). Oligonucleotide specificity was determined by a homology search within the human genome (BLAST, National Center for Biotechnology Information, Bethesda, MD) and confirmed by dissociation curve analysis. The oligonucleotide sequences are shown in Table 1. Oligonucleotides for TLR-4, SIRT1, peroxisome proliferator-activated receptor-gamma (PPAR-γ), PPAR-γ coactivator-1 alpha (PGC-1α), p53, FoxO1, FoxO3 and β-actin were designed using the computer software package Primer Express 2.0 (Applied Biosystems, Foster City, CA). All the oligonucleotides were synthesized by Invitrogen (Breda, The Netherlands). PCR was performed with SYBR Green in an ABI 7000 sequence detection system (Applied Biosystems) according to the manufacturer's guidelines.

Isolation of lymphocyte and nuclear extracts

Human lymphocytes were isolated from fresh whole blood by Ficoll-Paque PLUS (Pharmacia, 17-1440-02) and low-speed centrifugation as described previously (*26*). Nuclear and cytosolic extracts were isolated with a Nuclear and Cytoplasmic Extraction kit (Pierce Chemical, Rockford, IL). After the incubation period, lymphocytes were collected by centrifugation at 600 g for 5 min at 4°C. The pellets were washed twice with ice-cold PBS, followed by addition of 0.2 ml of cytoplasmic extraction buffer A and vigorous mixing for 15 seconds. Ice-cold cytoplasmic extraction buffer B (11 µl) was added to the solution. After vortex mixing, nuclei and cytosolic fractions were separated by centrifugation at 16,000 g for 5 min. The cytoplasmic extracts (supernatants) were stored at −80°C. Nuclear extraction buffer was added to the nuclear fractions (pellets), which were then mixed by vortex mixing on the highest setting for 15 s. The mixture was iced, and 15-s vortex was performed every 10 min for a total of 40 min. Nuclei were centrifuged at 16,000 g for 10 min. The nuclear extracts (supernatants) were stored at −80°C until use.

Immunoblotting

Proteins were separated by electrophoresis on sodium dodecyl sulphate–polyacrylamide gel. After the proteins had been transferred onto a PVDF membrane (Millipore, Bedford, MA), the blot was incubated with blocking buffer for 1 h at room temperature and then probed with primary antibodies (NF-κB, I-κB, PCNA and β-actin) overnight at 4°, followed by incubation with horseradish peroxidase-conjugated secondary antibody for 1 h. The bound immunoproteins were detected by an enhancer chemiluminescent assay (Amersham, Berkshire, UK).

Assay for cytokine concentrations

To test the cytokine concentrations, plasma was collected from whole blood after centrifugation at 2,500 g at 4°C for 10 min. Plasma was harvested and assayed for IL-1β, IL-6, IL-8, TNF-α, INF−γ and interferon-γ-induced protein 10-γ-induced protein 10 (IP-10) concentrations using commercial enzyme-linked immunosorbent assay (ELISA) kits (R&D Systems, Minneapolis, MN).

Table I. Primers used for real-time PCR

Gene	Sense	Anti-sense
TLR-4	5′-ATTTAAAGAAATTAGGCTTCATAAGCT-3′	5′-CCAAGAAGTTTGAACTCATGGTAA-3′
SIRT1	5′-TGTGGTAGAGCTTGCATTGATCTT-3′	5′-GGCCTGTTGCTCTCCTCAT-3′
p53	5′-GCCCACTTCACCGTACTAA -3′	5′-TGGTTTCAAGGCCAGATGT-3′
PGC-1α	5′-CCGCACGCACCG AAA-3′	5′-TCGTGCTGATATTCCTCGTAGCT-3′
PPAR-γ	5′-AGTGTGAATTACAGC AAATCTCTGTTTT-3′	5′-GCACCATGCTCTGGGTCA A-3′
FoxO1	5′-ATGGTCAAGAGCGTGCCC-3′	5′-GATTGAGCATCCACCAAG-3′
FoxO3	5′-TCTCCCGTCAGCCAGTCTAT-3′	5′-AGTCACTGGGGA ACTTGTCG-3′
β-actin	5′-CGGGAAATCGTGCGTGAC-3′	5′-TGCCCAGGAAGGAAGGCT-3′

Antioxidant enzyme activity measurement

To determine the antioxidant enzyme activity, plasma was collected from whole blood after centrifugation at 2,500 × g at 4°C for 10 min. Superoxide dismutase (SOD; Cell Biolabs, STA-340) and catalase activity (Cell Biolabs, STA-341) in the plasma was determined using an enzymatic assay from a commercial kit according to the manufacturer's instructions. Enzyme activity was converted to units per milligram of protein.

Hydrogen peroxide measurement

To determine the concentration of hydrogen peroxide, plasma was collected from whole blood after centrifugation at 2,500 × g at 4°C for 10 min. Hydrogen peroxide (Cell Biolabs, STA-344) in the plasma was determined using an enzymatic assay with a commercial kit according to the manufacturer's instructions. The concentration of hydrogen peroxide was converted to μM.

Statistical analyses

The data were expressed as the means ± SD. The experimental results before and after exercise intervention were analysed by paired-t test, and $P < 0.05$ was considered significant in our study.

Results

Subject characteristics and the pain relief after exercise intervention

A total of 30 patients with nonspecific low back pain were included in our study, and their mean age was 45 ± 3.25 years old. The mean visual analogue scale of pain was 6 ± 2.3 and 4 ± 1.5 before and after the exercise intervention, respectively. The P value was <0.01.

Exercise inhibits TLR-4 signalling in patients with nonspecific low back pain

Previous studies have suggested that exercise intervention reduces pain intensity and increases the quality of life in patients with low back pain (*9*, *27*). However, the possible molecular mechanisms and the translational regulation of exercise in low back pain patients remain largely unclear. Up-regulation of TLR-4 has been revealed in the development of rheumatoid arthritis and osteoarthritis (*14*). Therefore, we paid our attention on investigation whether exercise intervention effectively reduces TLR-4 expression in patients with nonspecific low back pain. Human blood mRNA was isolated, and real-time PCR was used to confirm our hypothesis. In Fig. 1A, we revealed the TLR-4 mRNA expression was largely inhibited after exercise in patients with nonspecific low back pain. TLR-4 activation causes interferon-γ (IFN-γ) up-regulation, and subsequent release of IP-10 is shown to play important roles in the regulation of pro-inflammatory events (*28*). In this study, we also confirmed that IFN-γ and IP-10 concentrations were significantly reduced after exercise in patients with nonspecific low back pain using ELISA assay (Fig. 1B and C). In order to further exam the molecular mechanisms of exercise for low back pain, we isolated the lymphocytes from low back pain patients and investigated the NF-κB activity by Western blotting assay. In Fig. 1D, we found the nuclear NF-κB p65 were repressed and the cytosolic I-κB were up-regulated after exercise intervention, indicating that our exercise programmes effectively reduces NF-κB activity in patients with nonspecific low back pain.

Exercise enhances SIRT1 expression in patients with nonspecific low back pain

SIRT1 is an important regulator that protects against oxidative events. A past study revealed that resveratrol, a kind of SIRT1 activator, was able to reduce levels of pro-inflammatory cytokines *in vitro* and showed analgesic potential in patients with discogenic low back pain (*21*). We thus hypothesized that exercise, like resveratrol, attenuates low back pain symptoms through the involvement of SIRT1 regulation. To confirm this, we compared the blood SIRT1 expression levels in patients with nonspecific low back pain before and after exercise intervention. In Fig. 2, we found that SIRT1 expression was significantly up-regulated after exercise in patients with nonspecific low back pain.

The downstream genes of SIRT1 are mediated by exercise intervention

SIRT1 was formerly suggested to counteract activation of the pro-apoptotic tumour suppressor p53 in response to genotoxic stress via deacetylation of lysine 379, thereby mitigating stress-induced cell death (*29*). Therefore, SIRT1 can promote stress resistance as well as mitigate pro-inflammatory responses (*30*). We therefore turned our attention to investigating p53 expression levels in patients with nonspecific low back pain. As shown in Fig. 3A, we found that p53 expression was decreased in the blood after exercise by real-time PCR assay. SIRT1 activation modulates downstream genes that also exert anti-inflammatory functions, such as the PPAR-γ and PGC-1α. Therefore, we used real-time PCR to investigate whether these genes were involved after exercise intervention. In Fig. 3B and C, our data indicated that the expression levels of PPAR-γ and PGC-1α were

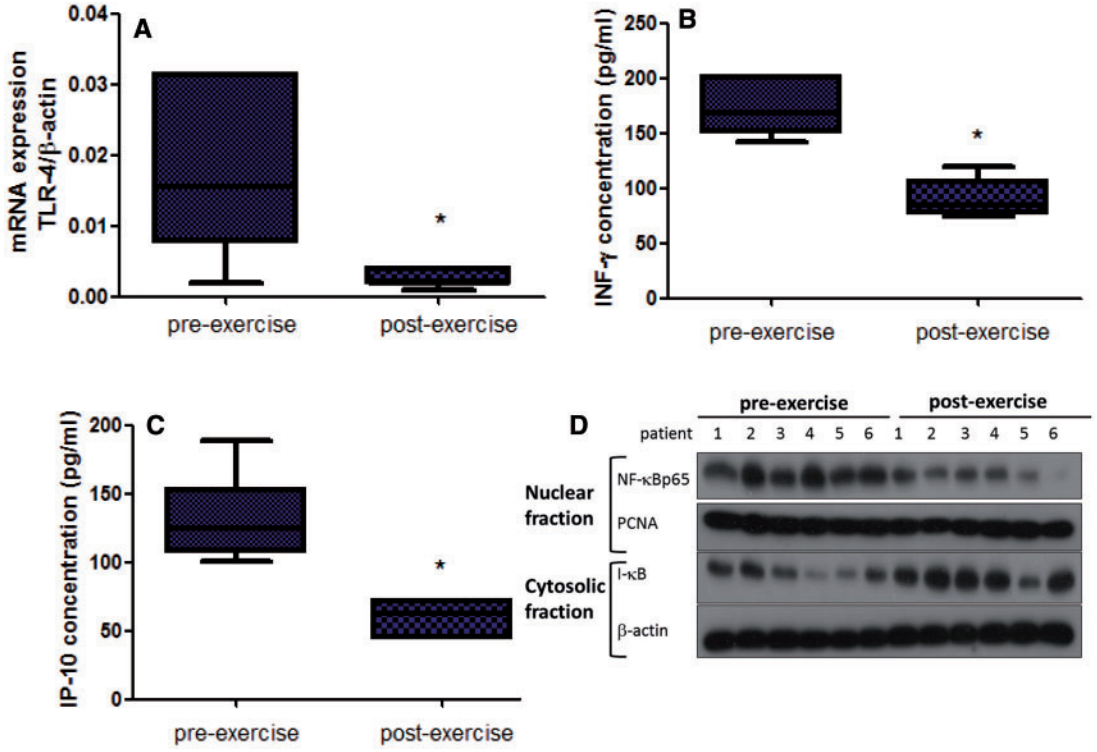

Fig. 1 Expression levels of TLR-4 signalling in patients with nonspecific low back pain. (**A**) Total blood mRNA was isolated from patients, and TLR-4 expression was investigated before and after exercise intervention using real-time PCR. (**B**) INF-γ and (**C**) IP-10 concentrations were tested by ELISA kits. (**D**) Nuclear NF-κB and cytosolic I-κB of peripheral blood lymphocytes were investigated by Western blotting assay.

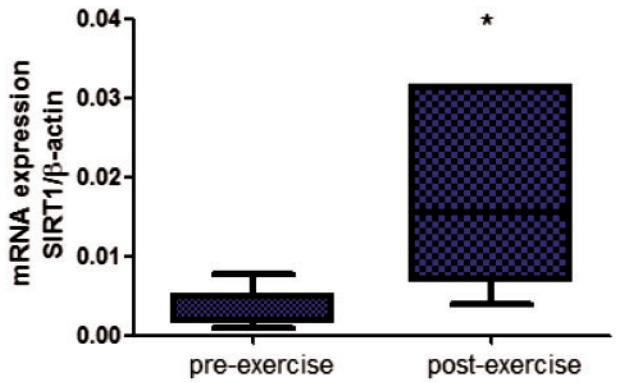

Fig. 2 Expression levels of SIRT1 in patients with nonspecific low back pain. Total blood mRNA was isolated from patients, and SIRT1 expression was investigated before and after exercise intervention using real-time PCR.

enhanced following exercise intervention in patients with nonspecific low back pain.

Expression levels of FoxO family after exercise in patients with nonspecific low back pain

The activity of FoxO family proteins is controlled by various posttranslational modifications, including phosphorylation, acetylation and ubiquitination, which in turn regulate their subcellular localization, protein–protein interactions and transcriptional activity (*31*, *32*). Indeed, the FoxO family plays important roles in regulating many cellular and biological processes, and is a critical mediator of cellular pathways. SIRT1 has been reported to deacetylate and activate three members of the FoxO family (*32*, *33*). Thus, we investigated the mRNA expression levels of FoxO proteins in patients with nonspecific low back pain. We reveal that FoxO1 and FoxO3 expression levels were strongly up-regulated in patients with nonspecific low back pain after exercise intervention (Fig. 4A and B).

Pro-inflammatory cytokine levels in patients with nonspecific low back pain

TNF-α, IL-1β, IL-6 and IL-8 are pro-inflammatory factors that are considered to be the primary regulators of inflammatory diseases. These pro-inflammatory markers induce NF-kB activation, and can be the potential target of treatment in these inflammatory diseases (*34*). Nevertheless, SIRT1 can inhibit IL-1β, IL-6, IL-8 and NF-kB activation under stress-induced pro-inflammatory conditions (*35*, *36*). We presumed that exercise-activated SIRT1 can inhibit the expression of these pro-inflammatory markers in patients with nonspecific low back pain. Plasma was isolated from the blood, and ELISA tests were used to examine the concentration of the pro-inflammatory markers. As shown in Fig. 5, the blood concentrations of IL-1β (Fig. 6A), IL-6 (Fig. 6B), IL-8 (Fig. 6C) and TNF-α (Fig. 6D) were down-regulated after exercise intervention in nonspecific low back pain patients.

Antioxidant enzyme activities are enhanced by exercise in patients with nonspecific low back pain

Several groups have suggested that activation of SIRT1 and FoxO inhibits pro-inflammatory responses via the induction of enzymes such as SOD and catalase

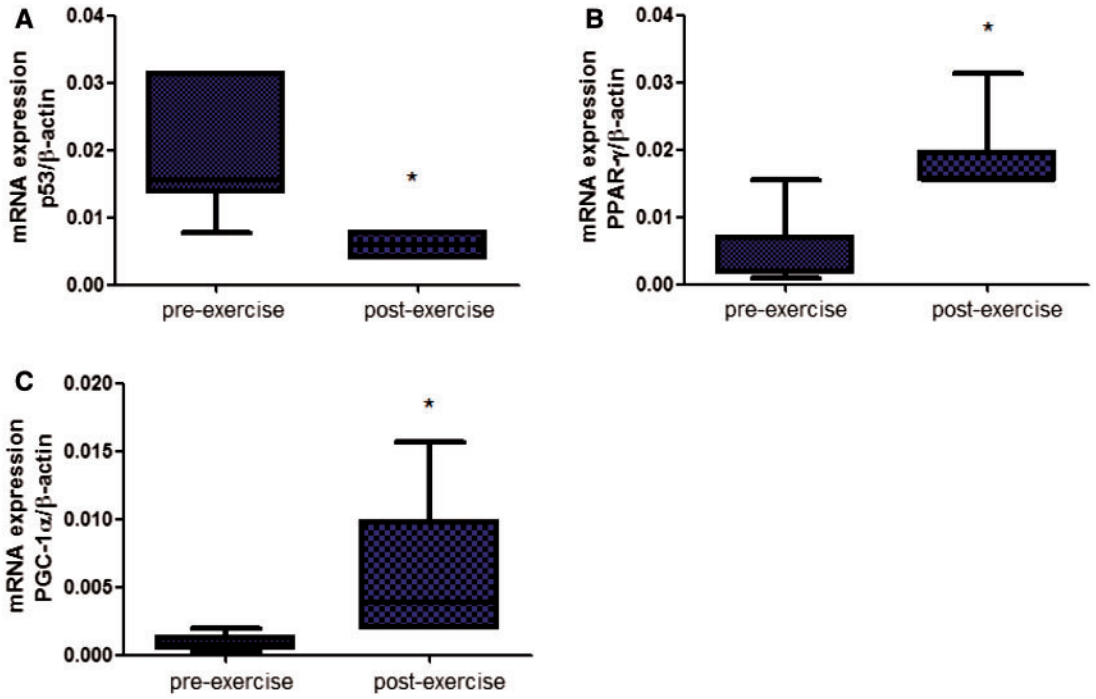

Fig. 3 Expression levels of SIRT1 downstream genes in patients with nonspecific low back pain. Total blood mRNA was isolated from patients, and the expression of (**A**) p53, (**B**) PPAR-γ and (**C**) PGC-1α was tested before and after exercise intervention using real-time PCR.

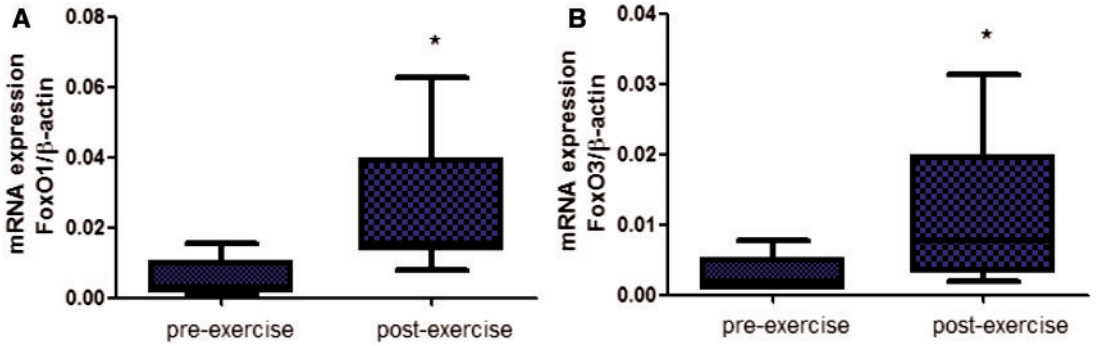

Fig. 4 Expression levels of FoxO proteins in patients with nonspecific low back pain. Total blood mRNA was isolated, and the expression of (**A**) FoxO1 and (**B**) FoxO3 was assessed before and after exercise intervention using real-time PCR.

(*37*, *38*). Therefore, we isolated plasma from whole blood and tested the antioxidant enzyme activities in all subjects. In Fig. 6A and B, we found that the activity of catalase and SOD were up-regulated after exercise intervention in patients with nonspecific low back pain.

Oxidative stress is decreased in patients with nonspecific low back pain after exercise intervention

The relationship between reactive oxygen species (ROS) and low back pain has been reported by previous studies (*39*, *40*). Battisti *et al.* (*39*) found that patients who suffered from low back pain showed higher systemic oxidative stress levels compared with control groups. Treatment using radical scavengers, such as SOD or alpha lipoic acid, improved functionality and reduced the use of analgesics in chronic low back pain patients (*39*). We presumed that exercise intervention can revert the elevated oxidative stress in patients with low back pain. As a result, we discovered that hydrogen peroxide concentrations were indeed diminished after our exercise intervention programmes (Fig. 7).

Discussion

Therapeutic exercises are often suggested to patients with low back pain because they address pain and musculoskeletal problems (*8*). The therapeutic effects of exercise are well described, such as increasing muscle power, increasing spine stability, enhancing quality of motion, reducing pain and decreasing disability (*41*). In this article, we demonstrate for the first time that exercise intervention provides a non-pharmacological inhibition of TLR-4 pathway and increase in blood SIRT1 expression levels in patients with nonspecific low back pain. Exercise intervention also functionally mitigates p53 expression and enhances

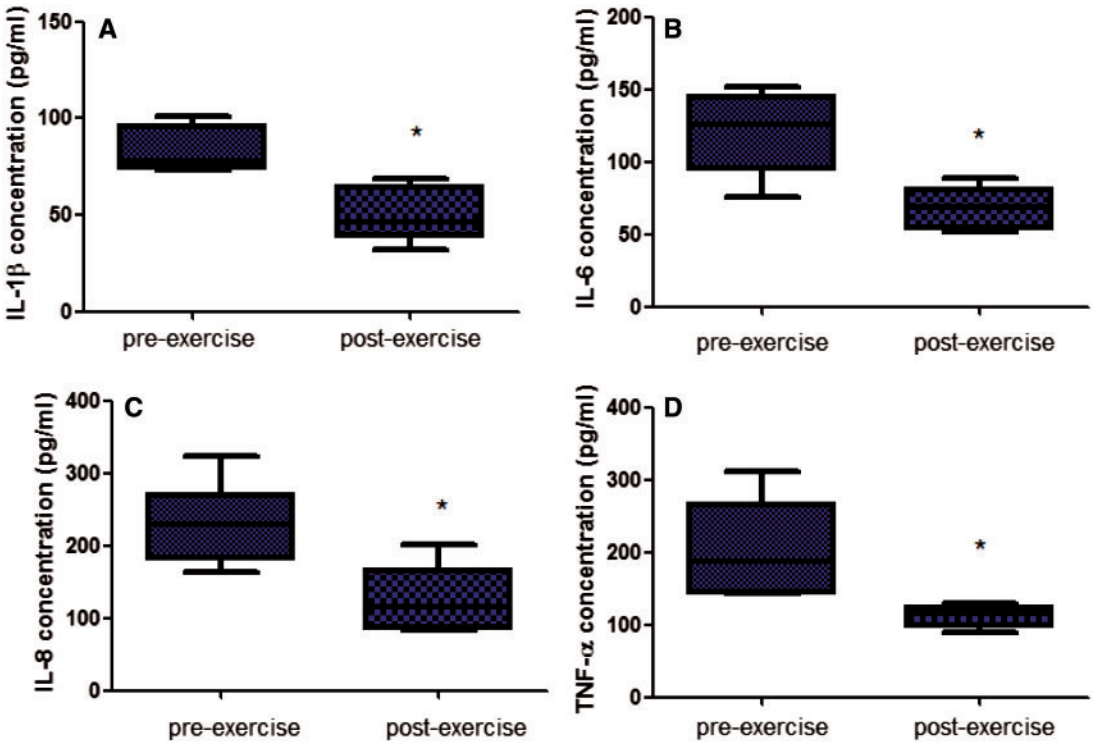

Fig. 5 Pro-inflammatory cytokine concentrations in patients with nonspecific low back pain. Blood plasma was isolated from patients. The concentration of (**A**) IL-1β, (**B**) IL-6, (**C**) IL-8 and (**D**) TNF-α were investigated before and after exercise intervention using ELISA kits.

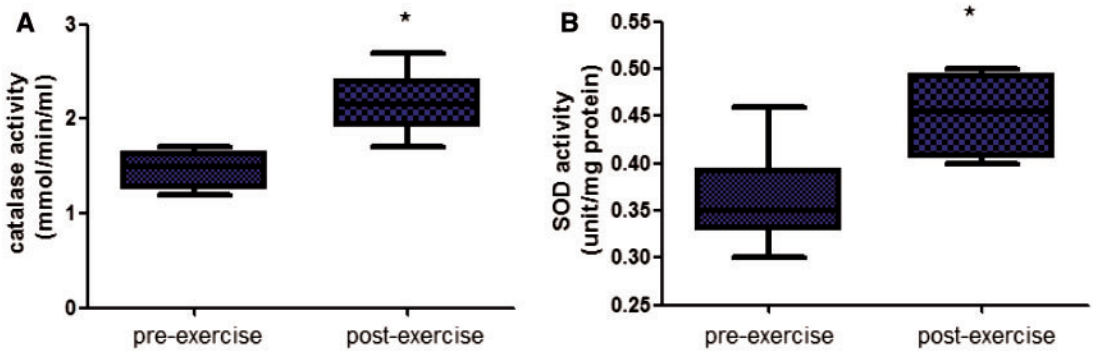

Fig. 6 Antioxidant enzyme activity in patients with nonspecific low back pain. Blood plasma was isolated from patients. The activity of (**A**) catalase and (**B**) SOD were investigated before and after exercise intervention using enzymatic assay kits.

PGC-1α, PPAR-γ and FoxO expression. In addition, our data suggest that exercise intervention effectively reduces pro-inflammatory cytokines in blood, thereby attenuating oxidative stress.

Chronic inflammation may play a key role in the regulation of low back pain symptoms (*42*). Muscle relaxation, inhibition of pro-inflammatory responses and increasing spine stability are the mainstream of treatment for low back pain (*43*). Activation of TLR-4 level has been found in diseases such as rheumatoid arthritis and osteoarthritis (*14*). Therefore, inhibition of TLR-4 may be a novel therapeutic intervention for patients with rheumatoid arthritis (*44*). Mitigation of TLR-4 function inhibits the severity of arthritis and causes down-regulation of IL-1 level in arthritic joints (*45*). Besides, TLR-4 triggered INF-γ and IP-10 activation are known to further activate the pro-inflammatory responses. It is well established that IP-10 antibodies can block clinical symptoms and sign for patients with arthritis (*46*). Endurance and resistance exercises have been suggested to repress TLR expression levels (*47*, *48*). The above findings in the past literatures is compatible with our study result that exercise could largely repress TLR-4 signalling, INF-γ activation and IP-10 release (Fig. 1).

Nuclear transcription factors of the NF-κB are repressed by I-κB proteins, which mitigate the activity of NF-κB by snaring NF-κB in the cell cytoplasm. The I-κB proteins are rapidly degraded under pro-inflammatory stimulation, thereby increasing the translocation of NF-κB into nuclear fraction (*49*). I-κB inhibition had been found in chronic pain model. Previously study suggested that pharmacological drug inhibits chronic pain and pro-inflammatory

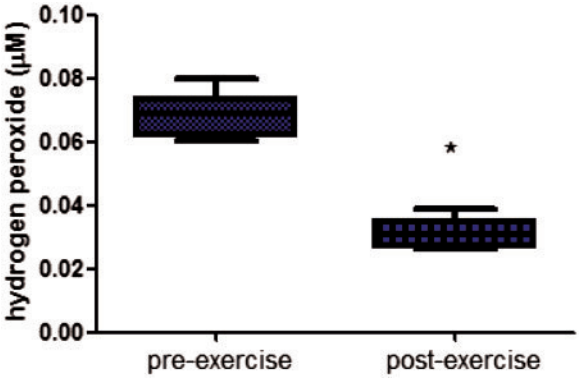

Fig. 7 ROS concentrations in patients with nonspecific low back pain. Plasma was isolated from whole blood. H_2O_2 concentrations were investigated using enzymatic assay kits.

events by enhancing the stability of cytosolic I-κB (*50*). PPAR agonist was shown to repress NF-κB activation through inhibition of I-κB degradation. Moreover, studies also confirmed that PPAR-γ reduces inflammation mediators by inhibition of NF-κB activation via reducing I-κB degradation in rheumatoid arthritis mice (*51*, *52*). Taken together, PPAR-γ/I-κB/NF-κB pathway might play an important role in regulation of chronic pain and chronic inflammation. Our data are harmonious with previous reports, suggesting that exercise up-regulates PPAR-γ level which repress I-κB degradation and NF-κB activation.

SIRT1 is known as a mammalian ortholog of yeast silent information regulator 2 and has been suggested to play a central role in the regulation of diseases such as diabetes, neurodegenerative and chronic inflammatory diseases (*53–55*). Schug *et al. (56)* reported that NF-κB is activated in SIRT1 knockout mice, thereby reinforce pro-inflammatory events. In our study, on the other hand, SIRT1 was proven to be activated with down-regulated NF-κB after exercise intervention, signifying that exercise can reduce inflammatory response *in vivo*, leading to relief of low back pain.

SIRT1 is a negative regulator of p53, which plays a central role in cell survival and chronic inflammatory diseases. The p53 gene can be activated by different stresses, such as hypoxia, chemotherapeutic drugs, radiation and oxidative stress (*57*, *58*). In normal conditions, p53 is destabilized and degraded and only small amounts of p53 can be detected in cells. However, genotoxic stress can stabilize p53, upon which it accumulates inside cells, resulting in cell cycle arrest, senescence and cell death (*59*). p53 has been proven to be largely related to inflammatory responses *in vivo* through reactive oxygen and nitrogen species, cytokines, infectious agents and major immune-regulatory pathways like NF-κB (*60*), and its activation can be inhibited by SIRT1 (*29*, *61*). The above studies correlated to our results that p53 expression was down-regulated along with NF-κB as a consequence of SIRT1 activation. Our findings are similar with previously reports from Dr. Radak group. They reveled exercise alters SIRT1 expression in skeletal muscle of rats (*62*), thereby mitigating oxidative stress, pro-inflammatory responses and enriching mitochondrial biogenesis in rats (*63–65*).

PGC-1α act as a transcriptional co-activator, controlling mitochondrial biogenesis via the activation of transcription factors that participate in the expression of mitochondrial proteins (*66*). A past literature has proven that musculoskeletal disorders and its sequelae are associated with PGC-1α dysregulation in muscle with a concomitant local or systemic inflammatory reaction, and NF-κB pathway plays a key role in linking metabolic and inflammatory programmes in muscle cells (*67*). On the other hand, SIRT1 can control the mitochondrial respiration chain, ATP production and ROS generation via modulating PGC-1α activity (*68*). Our study result is compatible with above literatures, revealing that PGC-1α was up-regulated secondary to NF-κB down-regulation and SIRT1 activation.

The FoxO transcription factors act at the interconnections between metabolic pathways inducible by many important signal transducers and mediators such as p53, Ikk-β, NF-κB, Akt, sirtuins, PTEN, etc (*69*). The FoxO family proteins have been linked to the control of immune system homeostasis in mammals (*70*). Moreover, multisystem inflammation may be caused by a lack of FoxO3a-related inhibition of NF-κB activity (*71*). In this study, we found that FoxO1 and FoxO3 expression levels were up-regulated after exercise intervention, thereby reducing the inflammatory responses and low back pain symptoms.

Yang *et al. (72)* reported that inflammatory cytokines may be used as biomarkers to monitor the physiological responses of low back pain during mechanical loading, and they found that plasma levels of IL-1β, IL-6 and TNF-α were increased following heavy lifting related low back stress. Another study revealed the associations between IL-1β and IL-6 concentrations and the number of key osteopathic lesions and between IL-6 and LBP severity at baseline. TNF-α concentration was revealed to change significantly after 12 weeks of exercise training (*73*). On the other hand, SIRT1 can mitigate IL-1, IL-6, IL-8 and NF-kB activation under stress-induced pro-inflammatory responses (*35*, *36*). Our result correlated to the literatures mentioned above that blood concentrations of IL-1, IL-6, IL-8 and TNF-α were down-regulated secondary to SIRT1 activation after exercise intervention, signifying that exercise can relieve low back pain via SIRT1-related inhibition of inflammatory responses.

The relationship between oxidative stress and low back pain has been previously postulated (*39*, *40*). Battisti *et al. (39)* showed that patients with low back pain had higher systemic oxidative stress levels compared with control groups. Moreover, treatment using radical scavengers such as SOD or alpha lipoic acid improved functionality and mitigated the use of analgesics in chronic low back pain patients. Taken together, enhancing antioxidant functions may be an effective intervention for chronic low back pain. Our results showed that exercise intervention could relieve low back pain symptoms by up-regulating antioxidant enzymes such as SOD and catalase, and thus

decreasing the concentration of hydrogen peroxide level in blood plasma.

The limitation of our study is that this is a cross-sectional design. A longitudinal study would be valuable to determine the long term effects of low back pain. Second, our study without a healthy control group, it is not clear if these molecular changes would have happened in subjects without low back pain.

Conclusion

In summary, we demonstrate that exercise intervention enhanced SIRT1 expression and up-regulated downstream genes including FoxO1, FoxO3, PGC-1α and PPAR-γ. TLR-4, NF-kB and p53 were then down-regulated, and the level of inflammatory cytokines including IL-1β, IL-6, IL-8 and TNF-α decreased. Furthermore, the activity of antioxident enzymes including SOD and catalase were elevated, and the oxidative stress measured as hydrogen peroxide was reduced. Exercise was proven to alleviate nonspecific low back pain by our study, and the mechanism behind was elucidated. However, SIRT1 has been shown to regulate other anti-inflammatory kinases such as AMP-activated protein kinase (AMPK) and AKT (*74*, *75*), and further work on the expression and phosphorylation status of these genes should be studied in the future to achieve further completeness of our study. Moreover, the levels of these markers in an age-matched control group without back pain are missed in this study, we will test the samples from non-LBP cases to support our hypothesis.

Ethical Review Committee Statement

This study was approved by Taipei Veterans General Hospital Institutional Review Board (IRB:2013-08-007A).

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Conflict of Interest

None declared.

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