ISSN 2618-1584 Vol. 5, Issue 1: 47-54, DOI: 10.25236/FMSR.2023.050109

Bioinformatics analysis based on the fitness effects of taijiquan in human immunity

Weimin Chen[1], Zhejian Wu[1], Xiaowen Zhang[2], Yiqing Guan[3], Wang Fei[4,*]

[1]*Guangzhou Sports Polytechnic, Guangzhou, China*
[2]*Dept. of Sports Nuitrition, Guangzhou Institute of Sports Science, Guangzhou, China*
[3]*Zhongshan City People's Hospital, Zhongshan, China*
[4]*Guangzhou Medical University, Guangzhou, China*
[*]*Corresponding author: 502854381@qq.com*

Abstract: *Objective: A systematic assessment of taijiquan fitness effects from an immunological perspective, with the aid of bioinformatics analysis. Methods: Bioinformatics as a means of analysis, including mainly: Gene Set Enrichment Analysis (GSEA analysis), GO pathway enrichment and PPI network integration to systematically analyse the target data sources (RNA-Seq data from Parkinson's patient population after 12 weeks of TCC and MET exercise) in the GEO database; Results: The TCC group significantly increased the expression of primary T cells relative to the MET group; it effectively increased the number of human CD8+ T and NK cells. human neutrophils were highly expressed in the MET group; TCC may activate more T cells, especially CD8+ and NK cells, by activating aerobic metabolic pathways in the body, whereas MET activates the expression of neutrophils and monocytes. TCC movement enhances the function of mitochondria in immune cells through respiration, which in turn further activates T cell proliferation through epigenetic modifications that move. Conclusion: TCC exercise is effective in boosting the body's acquired adaptive immunity, while MET exercise stimulates the activity of the body's innate immunity, making TCC more suitable for health and wellness, especially for the chronic patient population.*

Keywords: *Taijiquan Fitness, Immunity, Bioinformatics*

1. Introduction

In recent years, the relationship between tai chi (TCC) as a specific form of exercise intervention and immunity has received increasing attention from scholars in the life sciences. Taijiquan has developed into a "fitness symbol" and has become the main colour and theme of the sport, which is highly respected among the middle-aged and elderly. The survey found that Taijiquan has become one of the most popular sports among middle-aged and elderly women in Shanghai [1]. As a traditional Chinese martial art, Taijiquan is classified as a moderate intensity exercise due to its exercise intensity of 55% of the maximum oxygen intake [2], Long-term adherence to Taijiquan exercises can improve the body's balance, cardiovascular and respiratory functions [2-4]. It has been shown that [6], Regular 12 weeks of TCC exercise significantly improves functional mobility and regulatory T cell function in normal adult volunteers. It has also been shown that [5], Obese people who are physically inactive are at increased risk of pro-inflammation, and exercise induces the production of anti-inflammatory mediators in the body [7]. Vigorous exercise, which has been shown to promote a pro-inflammatory response in humans [8,9] and reduce adaptive immunity, increases the risk of upper respiratory tract infections [9,10]. Conversely, some studies have shown that moderate exercise can enhance T-cell function [11] and reduce respiratory infections [12]. The amount of exercise may be a key factor in determining whether the immune response is positive or negative [13].

2. Materials and methods

2.1 Materials (data source)

The gene expression profile of GSE124676 (Hu et al, published data, 2020) was downloaded from the GEO database (https://www.ncbi.nlm.nih.gov/gds) by searching using the keyword "Tai Chi". The data were obtained from PD patients who participated in tai chi or multimodal exercise training (MET) for 1 hour twice a week for 12 weeks. PBMC were isolated from whole blood and RNA-Seq was applied before and after training. the platform for GSE124676 is GPL17303, Ion Torrent Proton, and samples include 21 RNA-Seqs from PD patients practicing Tai Chi and 21 RNA-Seqs from PD patients practicing MET. the platform and series matrix files are downloaded in TXT format (GSE124676_ genes.fpkm_table.txt.gz, 2.6M). The R package was used to process the downloaded files and to convert and reject non-conforming data. Data were corrected, normalised and log2 transformed.

2.2 Methods

2.2.1 Gene set enrichment analysis (GSEA analysis)

GSEA was performed on the enhanced gene sets in the Taiji exercise group leukocyte RNA-seq. The gene sets interpreted by h.all.v8.0.symbols were selected for 1000 alignments. In GSEA version 3.0, the Molecular Signatures Database (MSigDB), data sets collapsed to gene symbols are considered 'false', while 'phenotype' indicates the type of alignment. GSEA was performed and the cut was: false discovery rate (FDR) $q > 0.25$, nominal $P < 0.05$.

2.2.2 GO pathway enrichment

Metascape (http://metascape.org) is an online tool for gene annotation, interactome and enrichment analysis and also facilitates comparative analysis of datasets across multiple independent and orthogonal experiments [43]. Metascape is used to identify pathways and perform enrichment analysis of transcription-associated CDKs and their closely related genes. Only terms with a minimum overlap value of 3, a p-value < 0.05 and a minimum enrichment value of 3 were included. The identified enriched ontology clusters were transformed into a network layout. Protein-protein interaction enrichment analysis was performed on the BioGrid, InWeb_IM and OmniPath databases. The MCODE algorithm was then applied to the protein interaction network to identify protein-connected dense modules.

2.2.3 PPI network integration

The STRING database (http://string-db.org/) is a software system that is typically used to identify known proteins and predict protein interactions. The results of this database are derived from experimental data, databases, text mining and predictive bioinformatics data In addition, the core of Cytoscape software is the network. Each node is a gene, protein or molecule and the connections between the nodes represent interactions between these biomolecules and can be used to identify interactions and pathway relationships between proteins encoded by genes enhanced in the Taiji Movement Group leukocyte RNA-seq. The corresponding proteins in the central nodes may be core proteins or key candidate genes with important physiological regulatory functions.

3. Results

To investigate in depth the effect of TCC practice and MET on the transcriptome, we entered the (FPKM) values of 21 transcriptomes of Parkinson's patients practicing TCC in transcriptome 1 and 21 transcriptomes of Parkinson's patients trained in MET in transcriptome 2, for a total of 26,111 gene transcripts, into GSEA software, and used the gene expression profile data of various single cells in c8 classes therein to compare and observe the transcriptomes in transcriptome 1 and transcriptome 2 The main cell types with differential expression.

3.1 Analysis of differences in initial T cell expression

As shown in Figure 1A, the main differences between TCC transcriptome 1 and MET transcriptome 2 showed that the former had a more significant expression of initial T cells than the latter (NES=2.55, p-value=0); as shown in Figure 1B, the 201 genes upregulated in transcriptome 1, relative to transcriptome 2, belonged to the bone marrow-derived initial T cells in the single cell sequencing analysis. The marker genes are: RHOH, BCL2, CAMK4 and other 201 genes. Analysis by Matescape software showed that the main associated biological processes are: T cell differentiation, T cell activation, T cell aggregation, T cell signalling pathway, TNF-alpha/NF-kappa B signalling pathway, and VEGFA-VEGFR2 signalling pathway, see Figure 1c, d.

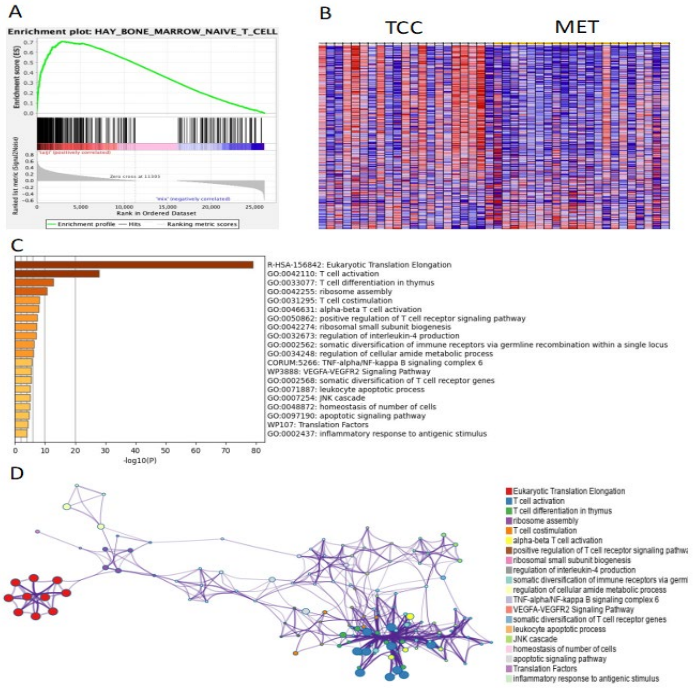

Figure 1: A: Curve plot of cell type enrichment annotations in the TCC and MET groups; B: Heat map of differential expression of initial T cell-associated genes in the TCC and MET groups, with high expression indicated in red and low expression in blue; C: Bar graph of functional annotations of initial T cell-associated genes in the TCC group, with colours indicating p-values; D: Net relationship between individual functions in Figure C.

3.2 Analysis of CD8+ T and NK cell differences

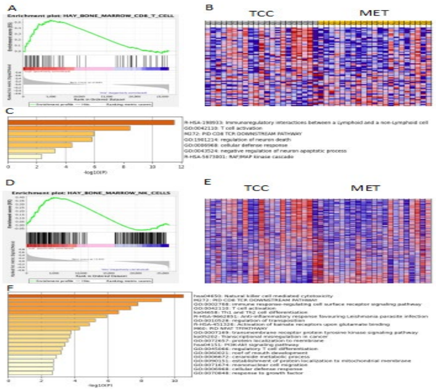

Figure 2: A, D: Curves of cell type enrichment annotations in the TCC and MET groups; B, E: Heat map of differential expression of CD8+T/NK cell-related genes in the TCC and MET groups, with high expression indicated in red and low expression in blue; C, F: Bar graphs of functional annotations of related genes in the TCC group, with colours indicating p-values.

Transcriptome 1 of TCC group, relative to MET transcriptome 2, had more CD8+ T cells (NES=1.65,p-value=0.001), as shown in Figure 2A, where 201 genes were upregulated in transcriptome 1 relative to transcriptome 2, belonging to bone marrow-derived CD8+ T cells in single cell sequencing analysis. The main biological processes involved are: T cell activation, immunoregulatory interactions between lymphoid and non-lymphoid cells, CD8 TCR downstream pathway, negative regulation of neuronal apoptotic processes, MAPK1 / MAPK3 signalling, T cell aggregation, T cell signalling pathway, and TNF-alpha/NF-alpha signalling. TNF-alpha/NF-kappa B signalling pathway, VEGFA-VEGFR2 signalling pathway, see Figure 2c. TCC transcriptome 1, with more NK cells relative to MET transcriptome 2 (NES=1.42,p-value=0), is shown in Figure 2A, where transcriptome 1 is upregulated relative to transcriptome 2 for 130 genes that are part of the single cell sequencing analysis of bone marrow-derived NK cells. The marker genes are: FASLG, CD247, GZMB and 130 other genes, and the main associated biological processes are: natural killer cell-mediated cytotoxicity, CD8 TCR downstream pathway, immune response regulatory cell surface receptor signalling pathway, T cell activation, Th1 and Th2 cell differentiation,as seen in Figure 2D.

3.3 Differential analysis of neutrophil expression

The MET group, transcriptome 2, had more neutrophil cells relative to TCC transcriptome 1 (NES=-2.40, p-value=0), as shown in Figure 3A. 265 genes were upregulated in transcriptome 2 relative to transcriptome 1, which belonged to bone marrow-derived neutrophils in the single cell sequencing analysis. The 265 genes, including ACTB, CD14, CEACAM4 and other markers, were analysed by Matescape software and showed that the main relevant biological processes were: neutrophil degranulation, regulation of vesicle-mediated transport, phagocytosis, regulation of defence responses, I-kappaB kinase/ NF-kappaB signalling, vitamin D receptor pathway, macrophage activation, etc. See Figure 3c. MET transcriptome 2, with more monocytes (MONOCYTE) cells relative to transcriptome 1 (NES=-2.17, p-value=0), as shown in Figure 3A. 118 genes upregulated in transcriptome 2 relative to transcriptome 1, belonging to bone marrow-derived monocytes in single cell sequencing analysis. The marker genes are: AIF1, LYN, MYD88 and 118 other genes. The main relevant biological processes, as seen by Matescape software analysis, are: activation of phagocytosis by bone marrow leukocytes, leukocyte wandering, regulation of cytokine production, inflammatory response, positive regulation of hydrolase activity, etc., see Figure 3f

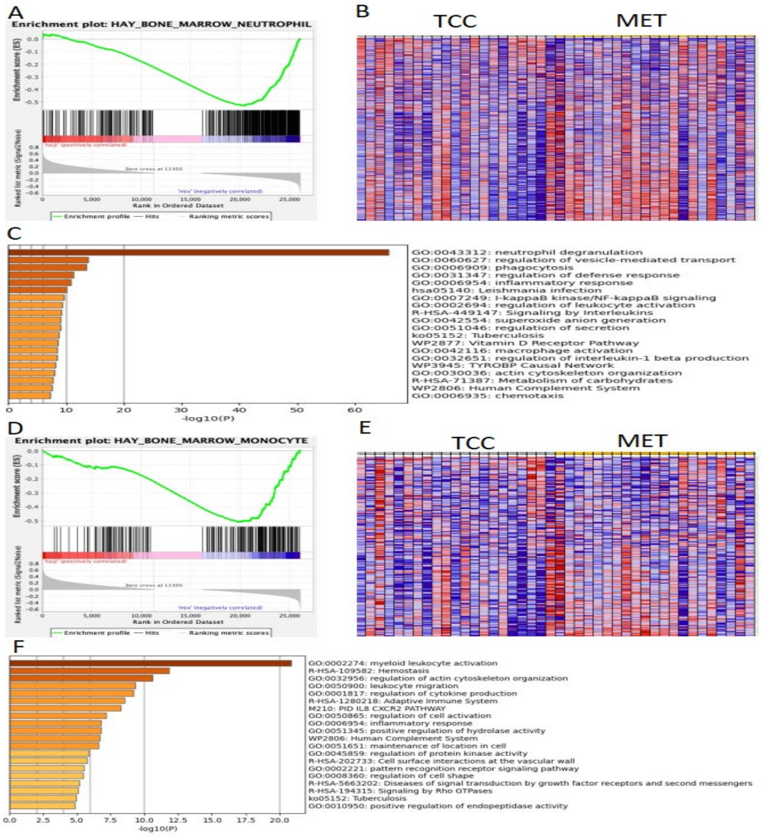

Figure 3: A, D: graphs of cell type enrichment annotations in the TCC and MET groups; B, E: heat map of differential expression of genes associated with the TCC and MET groups being granulocyte/monocyte cells, with high expression indicated in red and low expression in blue; C, F: bar graphs of functional annotations of genes of interest in the MET group, with colours indicating p-values.

3.4 Analysis of differential expression of fatty acid metabolism and oxidative phosphorylation-related genes

After analyzing the two transcriptomes, TCC and MET, and the cell types in which they were predominantly expressed, the relevant biological pathways of the transcriptomes were further analyzed, and the results showed that the expression of fatty acid metabolism and oxidative phosphorylation-related genes in the TCC transcriptome was significantly higher than that in the MET group, as shown in Figure 4a, d. These fatty acid metabolism-related genes upregulated in the TCC group may be regulated by SP1, PPARA, FOS,STAT1,JUN genes, as shown in Figure 4b. The oxidative phosphorylation-related genes up-regulated by TCC may be regulated by DMRT1, DLX6.TAF9B, NCOA2, ZNF197 genes, as shown in Figure 4.

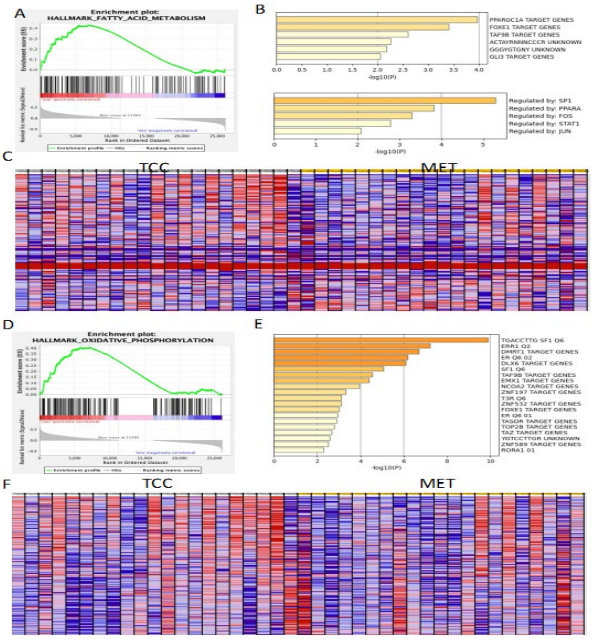

Figure 4: A, D: Curves of biological function enrichment annotations in the TCC and MET groups; B, E: Possible regulatory factors of related genes in the TCC group, colours indicate p-values; C, F: Heat map of differential expression of related genes in the TCC and MET groups, high expression is indicated in red and low expression is indicated in blue.

3.5 Differential analysis of IL6 pro-inflammatory factor expression

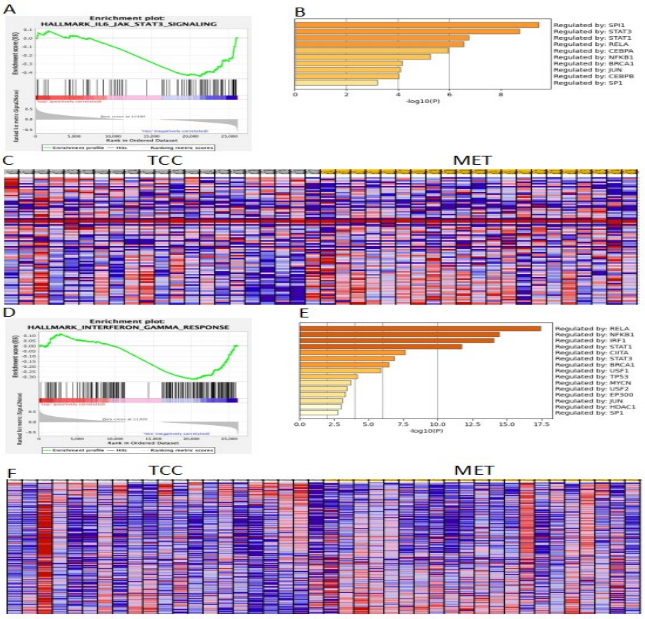

Figure 5: A, D: Curves of biological function enrichment annotations in the TCC and MET groups; B, E: Possible regulatory factors of related genes in the MET group, colours indicate p-values; C, F: Heat map of differential expression of related genes in the TCC and MET groups, high expression is indicated in red and low expression is indicated in blue.

The expression of genes related to IL6_JAK_STAT3 signaling pathway and interferon signaling pathway was significantly higher in MET group than in TCC, as shown in Figure 5a,d. The genes related to IL6_JAK_STAT3 signaling pathway upregulated in MET group might be regulated by SPI1, STAT3, STAT1, RELA, CEBPA, etc., as shown in Figure 5b; the genes related to interferon signaling pathway upregulated in MET group might be regulated by RELA, NFKB1, IRF, STAT1, CITA and other genes, as shown in Figure 5e. Based on the results of the above analysis, it can be speculated that TCC may activate the mitochondria-related fatty acid metabolism, oxidative phosphorylation pathway through more deep breathing, thus activating more T cells, especially CD8+ and NK cells, while MET may activate IL6_JAK_STAT3 signalling pathway and interferon signalling pathway through more anaerobic exercise to produce metabolites such as lactate, thus more activation of neutrophils and monocytes associated with inflammatory responses.

3.6 Analysis of epigenetic differences

We constructed a PPI network of proteins encoded by taiji motility group upregulated genes and identified seven closely related genes: CDK1, CDK2, PCNA, CCNE1, CDC20, HSP90AA1, and MCM4. The proteins encoded by these genes are key nodes in the PPI network, as shown in Figure 6a. Tai Chi exercise enhances mitochondrial function in immune cells through respiration, which in turn further activates T cell proliferation and movement through epigenetic modifications. The molecular mechanisms involved deserve further exploration.

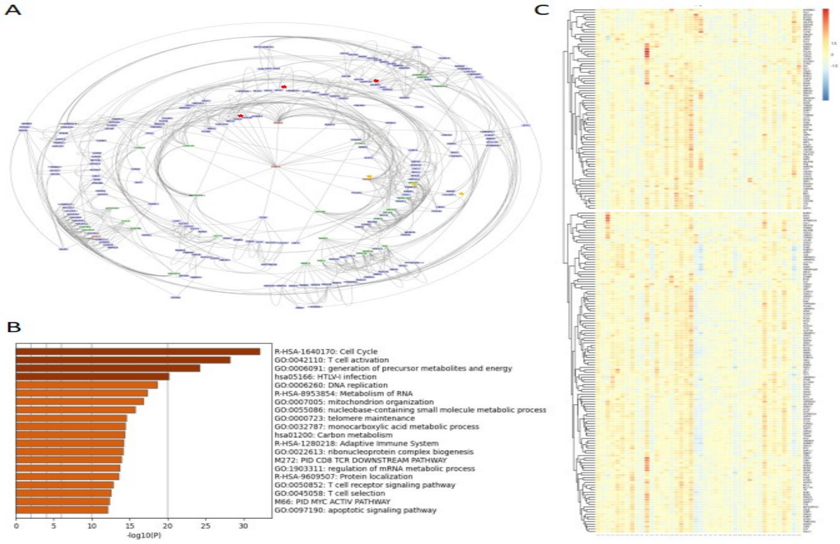

Figure 6: A: A mesh map of the relationship between the edited proteins of the up-regulated genes in the TCC group, red and yellow stars indicate epistasis modification-related genes and their associated proteins; B: Heat map of the differential expression of the up-regulated genes and MET in the TCC group, high expression is indicated in red and low expression is indicated in blue; C: Annotated biological functions associated with the up-regulated genes in the TCC group, colours indicate p-values.

4. Conclusion

TCC significantly increases the expression of primary T cells compared to MET. TCC is effective in increasing the number of human CD8+ T and NK cells, thus significantly improving the human acquired adaptive immune capacity. TCC may activate more T cells, especially CD8+ and NK cells, by activating aerobic metabolic pathways in the body, whereas MET may activate more neutrophils and monocytes associated with inflammatory responses by producing metabolites such as lactate through more anaerobic exercise. Epigenetic results show that Tai Chi exercise enhances the function of mitochondria in immune cells through respiration, which in turn further activates T cell proliferation through epigenetic modifications that move.

5. Discussion

Taijiquan, as a typical representative of traditional Chinese martial arts, focuses on the unity of heaven and man, emphasizing both the coordination and unity of the limbs and the close cooperation of breathing and intention, and its emphasis on the health concept of internal nourishment, external training and external concurrent emphasis, "issuing from within to the outside, and converging from the outside to the inside, cultivating both inside and outside". The form is the bearer of life, the qi is the energy of life, and the god is the dominant force of life, thus achieving: "The form and the god are close to each other, and both the surface and the inside are helpful" [14]. During the practice of Taijiquan, its gross oxygen uptake does not exceed 55%, so TCC is classified as a moderate exercise intensity program [15]. Several studies have shown that regular TCC exercise benefits balance, cardiovascular and respiratory function, flexibility, muscle strength and psychological effects [16-18].

In acute exercise, neutrophil concentrations in the body remain progressively elevated after cessation of exercise and continue until 6 hours post-exercise [19]. The increase in neutrophils is most likely caused by increased levels of catecholamines and biophysical shear stress in the vascular bed, leading to the release of neutrophils to adhere to the endothelium, a response that is largely dependent on the release of mature neutrophils from the limbic pool (liver, lung, spleen and bone marrow), possibly mediated by cortisol and/or other signals (e.g. growth hormone) [20-23]. This response is highly dependent on the intensity of exercise and has a positive dose-response relationship. In addition to an increase in numbers, neutrophils are also activated and/or initiated by acute exercise, characterised by functional adaptation [24]. High-intensity exercise, marathon and laboratory exercise tests have all shown to delay neutrophil apoptosis [25]. Studies have shown that post-exercise isolated serum (simulating in vitro exercise conditions) delays apoptosis of resting neutrophils in vitro, highlighting per-cell changes in neutrophils following exercise. In contrast to these positive effects, strenuous and prolonged vigorous exercise may negatively affect neutrophil function [26]. This limitation of neutrophil function and viability caused by strenuous exercise is thought to be widely dependent on the training status of the subject and can be counteracted by moderate exercise training [27,28]. Following acute strenuous exercise, haematopoietic stem and progenitor cells can be mobilised into the circulation of humans and rodents [29]. A progressive moderate-to-vigorous exercise programme did not have a negative feature, but was beneficial to the IL-2/natural killer cell system.

The data analysed in this study are consistent with the relevant findings reported in the reviewed literature, further suggesting that the traditional Chinese health maintenance practice, Taijiquan, has significant advantages in strengthening the body, especially in improving the body's acquired immunity. In contrast, other forms of exercise, anaerobic resistance training, including aerobic prolonged exercise all trigger the switch on of the body's innate immunity, yet overuse of innate immunity can have adverse consequences on the body such as pro-inflammatory reactions. Of course, this study involved data from a group of 12 weeks only, and there is a lack of data on long-term exercise. Therefore, in the next stage, we will select people who have been practicing taijiquan programs for a long period of time (e.g., more than 5 years) and further analyse specific data on innate and acquired immunity in this group, so as to gain a comprehensive understanding of the fitness effects of taijiquan on the human body in terms of enhancing acquired immunity.

Acknowledgements

Supported by: Project on Construction of Industrial Technology Collaborative Innovation Center of Guangzhou City in 2023

References

[1] Tan YT, Gao YT, Liu DK et al. Physical activity patterns of middle-aged and elderly women in Shanghai[J]. Chinese Chronic Disease Prevention and Control, 2007, 15(4): 309-313.
[2] Wang C, Collet JP, Lau J: The effect of TCC exercise on health outcomes in patients with chronic conditions: a systematic review. Arch Intern Med 164:493– 501, 2004
[3] Lu WA, Kuo CD: The effect of Tai Chi Chuan on the autonomic nervous modulation in older persons. Med Sci Sports Exerc 35:1972–1976, 2003
[4] Tsang WW, Hui-Chan CW: Effect of 4- and 8-wk intensive Tai Chi training on balance control in the elderly. Med Sci Sports Exerc 36:648 – 657, 2004
[5] Yeh SH, Chuang H, Lin LW, Hsiao CY, Eng HL: Regular Tai Chi Chuan exercise enhances

functional mobility and CD4CD25 regulatory T cells. Br J Sports Med 40:239 –243, 2006

[6] Bruunsgaard H: Physical activity and modulation of systemic low-level inflamation. J Leukoc Biol 78:819 – 835, 2005

[7] Peterson AM, Pederson BK: The antiinflammatory effect of exercise. J ApplPhysiol 98:1154 –1162, 2005

[8] Suzuki K, Nakaji S, Yamada M, Liu Q, Kurakake S, Okamura N, Kumae T, Umeda T, Sugawara K: Impact of a competitive marathon race on systemic cytokine and neutrophil responses. Med Sci Sports Exerc 35:348 –355, 2003

[9] Suzuki K, Nakaji S, Yamada M, Totsuka M, Sato K, Sugawara K: Systemic inflflammatory response to exhaustive exercise: cytokine kinetics. Exerc Immunol Rev 8:6 – 48, 2002

[10] Nieman DC: Current perspective on exercise immunology. Curr Sports Med Rep 2:239 –242, 2003

[11] Shore S, Shinkai S, Rhind S, Shephard RJ: Immune responses to training: how critical is training volume? J Sports Med Phys Fitness 39:1–11, 1999

[12] Davis JM, Murphy EA, Brown AS, Carmichael MD, Ghaffar A, Mayer EP: Effects of moderate exercise and oat beta-glucan on innate immune function and susceptibility to respiratory infection. Am J Physiol Regul Integr Comp Physiol 286:R366 – R372, 2004

[13] Shore S, Shinkai S, Rhind S, et al. Immune responses to training: how critical is training volume? J Sports Med Phys Fitness 1999;39:1–11.

[14] An Nan, Wang Songtao, Wang Anli et al. Research on the appropriate intensity of fitness exercise for middle-aged and elderly women[J]. Sports Science. 2005, 25(8): 62-66.

[15] Wang C, Collet JP, Lau J. The effect of TCC exercise on health outcomes in patients with chronic conditions: a systematic review. Arch Intern Med 2004;164:493–501.

[16] Chan SP, Luk TC, Hong Y. Kinematic and electromyographic analysis of the push movement in tai chi. Br J Sports Med 2003;37:339–44.

[17] Li JX, Hong Y, Chan KM. Tai chi: physiological characteristics and beneficial effects on health. Br J Sports Med 2001;35:148–56.

[18] Li JX, Hong Y, Chan KM. Effects of long term Tai Chi practice and jogging exercise on muscle strength and endurance in older people. Br J Sports Med 2006;40:50–4.

[19] McCarthy, D. A. and Dale, M. M. (1988) 'The leucocytosis of exercise. A review and model.', Sports medicine (Auckland, N.Z.). New Zealand, 6(6), pp. 333–363.

[20] McCarthy and Dale, 1988;Kappel, M. et al. (1993) 'Effects of an Acute Bolus Growth Hormone Infusion on the Human Immune System', Horm Metab Res, 25(11), pp. 579–585. doi: 10. 1055/s-2007-1002181., 1993

[21] Pyne, D. (1994) 'Regulation of neutrophil function during exercise.', Sports medicine (Auckland, N.Z.), 17(4), pp. 245–58. doi: 10.2165/00007256-199417040-00005.

[22] Summers, C. et al. (2010) 'Neutrophil kinetics in health and disease', Trends in Immunology. Elsevier Science Ltd, 31(8), pp. 318–324. doi: 10.1016/j.it.2010.05.006.

[23] Brown, W. M. C. et al. (2015) 'A Systematic Review of the Acute Effects of Exercise on Immune and Inflammatory Indices in Untrained Adults', Sports Medicine - Open. Sports Medicine - Open, 1(1), p. 35. doi: 10.1186/s40798-015-0032-x.

[24] Pyne, D. (1994) 'Regulation of neutrophil function during exercise.', Sports medicine (Auckland, N.Z.), 17(4), pp. 245–58. doi: 10.2165/00007256-199417040-00005.

[25] Mooren, F. C. et al. (2012) 'Exercise delays neutrophil apoptosis by a G-CSF-dependent mechanism', Journal of Applied Physiology, 113(7), pp. 1082–1090. doi: 10. 1152/ japplphysiol. 00797. 2012.

[26] Pyne, 1994;Pyne, D. B. et al. (1995) 'Effects of an intensive 12-wk training program by elite swimmers on neutrophil oxidative activity.', Medicine and science in sports and exercise. United States.

[27] Su, S. H., Jen, C. J. and Chen, H. ing (2011) 'NO signaling in exercise training-induced antiapoptotic effects in human neutrophils', Biochemical and Biophysical Research Communications. Elsevier Inc., 405(1), pp. 58–63. doi:10.1016/j.bbrc.2010.12.123.

[28] Syu, G. Da, Chen, H. I. and Jen, C. J. (2011) 'Severe exercise and exercise training exert opposite effects on human neutrophil apoptosis via altering the redox status', PLoS ONE, 6(9). doi: 10. 1371/ journal. pone.0024385.

[29] Emmons, R. et al. (2016) 'Acute exercise mobilizes hematopoietic stem and progenitor cells and alters the mesenchymal stromal cell secretome', Journal of Applied Physiology, 120(6), pp. 624–632. doi: 10.1152/japplphysiol.00925.2015.