

Anti-inflammatory effect on genes expression after four days of Qigong training in peripheral mononuclear blood cells in healthy women

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Abstract

Introduction. Some studies have shown the influence of Qigong on gene expression in different cells, but there is little data associated with the influence of this kind of therapy on genes expression in peripheral monocellular blood cells.

Objective. The aim of this study was to evaluate changes in the expression of genes associated with cellular stress response in peripheral mononuclear blood cells (PMBC) in healthy women.

Materials and method. The experiment took place at the Japanese Martial Arts Centre “DOJO” in Stara Wieś, Poland, conducted over the course of a 4-day qigong training session. To evaluate the genes effect of this training, blood samples were taken before and after the training period. This experiment involved 20 healthy women (aged 56.2±9.01, body height 164.8±6.5 and mass 65.5±8.2). To determine the expression of *HSF-1*, *HSPA1A*, *NF-kB*, *IL10* and *CCL2* mRNA, 3 ml of venous blood was collected. The blood samples were placed in tubes allowing for separation (BD Vacutainer CPT™) before and after the 4 days of qigong training. Isolated PMBC were used to determine gene expression using real-time qRT-PCR (quantitative reverse transcription polymerase chain reaction).

Results. Significant decreases in *NF-kB* and *CCL2* mRNA and increases in *IL10*, *HSF1* and *HSPA1A* m-RNA were detected after 4 days of qigong training. The obtained findings suggest that qigong caused a reduction in the inflammatory and intensified anti-inflammatory gene expression, as well as a higher expression of *HSF-1* and *HSPA1A*.

Conclusion. The adaptive response to qigong training was similar to the adaptive response to physical activity and was detected through gene expression in PMBC. Furthermore, this kind of training is especially indicated for women because of their higher susceptibility to psychosocial stress when compared to men.

Key words

qigong, genes expression, heat shock protein, inflammation

INTRODUCTION

Modern lifestyles represent an enormous challenge for our bodies. Time constraints, stress, lack of sleep, and environmental pollution are all factors exerting pressure on the nervous, endocrine, and cardiovascular systems, including gene activity. Qigong is one method of exercise belonging to the category of mind-body therapy (MBT). According to Astin et al. [1], MTB can influence human

health and is an important part of unconventional medicine. Current subject literature shows a plethora of data that has demonstrated the positive impact of MBT on human health [2–4]. Recently, the healthy effects of qigong have been better documented regarding its application in maintaining wellness [5, 6]. Loucks et al.[7] reported that this type of exercise has a positive impact on the cardiovascular system and is helpful in smoking cessation, as well as the stabilization of body mass. Types of exercise, including qigong, have been introduced in the training and rehabilitation of older people. For this group, qigong can improve balance, agility, muscular strength and reduce the risk of falls [4, 5]. It is also useful in reducing the symptoms of restless legs syndrome [8]. There are some reports on MBT in which the cytotoxic effect on

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cancer cells has been analysed, including the tumour cells of the pancreas [9] and breast cancer cells [10], as well usefulness in stuttering therapy [11].

Psychosocial stress results in increased inflammatory markers in the blood, i.e., interleukin 6 (IL6), CCL2, C-reactive protein (CRP), E-selectin and molecule of intercellular adhesion-1 [12, 13]. Therefore, the researchers' interest was also related to the influence of MBT, including qigong, on the reduction of inflammatory gene activity [14–17]. The most popular experiments are those in which proinflammatory interleukins are detected. Li et al. [18] reported that qigong training can enhance the immune response, resolution of inflammation, and downregulation of cellular metabolism in neutrophil transcription. Furthermore, in a study involving patients with early stage breast cancer, Antoni et al. [19] reported a decrease in GATA transcription factors and NF- κ B/Rel activity, and an increase in the interferon response to glucocorticoid receptor activity. A decrease in NF- κ B activity in leukocytes after MBT was also indicated by Black et al. [20], Creswell et al. [21] and Bower et al. [22]. Moreover, Kaliman et al. [23] reported a decrease in the proinflammatory cytokine gene expression after mindful meditation in peripheral blood mononuclear cells.

In the subject literature, studies of genes associated with apoptosis following qigong are relatively rare. Sharma et al. [24], along with Sudarshan Kriya, reported the upregulation of the expression of genes related to survival from oxidative stress. Despite data showing the positive impact of qigong training [18], little is known about the impact of this training on gene expression in PMBC associated with stress response.

OBJECTIVE

The aim of this study was to evaluate changes in the expression of *HSF1*, *HSPA1A*, *IL6*, *IL10*, *CCL2* and *NF- κ B* mRNA in healthy women. It was assumed that the pro-health effect would be similar to those obtained by physical exercise, noticeable through decreases in *NF- κ B*, *IL6* and *CCL2* mRNA, increases in *IL10* mRNA, and increases in *HSF1* and *HSPA1A* mRNA. To the best of the authors' knowledge, *CCL2* expression after MBT has not been subsequently evaluated, although it is important in the pathogenesis of several diseases, such as psoriasis, rheumatoid arthritis and atherosclerosis [25].

MATERIALS AND METHOD

Participants and ethics. A total of 20 healthy women aged 56.2 ± 9.01 , body height 164.8 ± 6.5 , body mass 65.5 ± 8.2 and body mass index (BMI) 24.2 ± 3.1 , participated in the experiment. All subjects had a normal health status over the course of the 2 months of the study (no drugs, alcohol, nicotine, negative medical history, etc.). Additionally, they were subject to a physical examination and gave consent for their participation in the experiment.

The study was approved by the Bioethics Committee for Clinical Research at the Regional Medical Chamber in Gdańsk, Poland, and conducted according to the Declaration of Helsinki. All participants gave their written consent to participate in the study and were informed about the purpose and test procedures. Additionally, they were made aware of

the possibility of a withdrawal of consent at any time for any reason.

Qigong training. During 4 days of training, all participants were grouped together, were similarly nourished, and had similar circadian rhythms. Everyone was subjected to identical training and performed a qigong exercise programme under instructor guidance. All of the women had a normal rest value of heart rate (HR between 70–90), and during the training programme they were obliged to ensure that the HR did not exceed more than 30% of the resting value. Each day, participants were trained for 8 hours (4 hours for a system called “5 hand movements,” and 4 hours of meditation). The “system called 5 hand movements or 6 secret wards” exercises focused on the performance of low, soft movements, with deep relaxation of the muscles and slow, deep, breathing focusing on suitable circulation circuits: liver, lung, heart, kidney, spleen, and pancreas. Meditations were performed in either the standing position “big tree” (1–2 hours) or sitting position (2–3 hours) while participants focused on selected points called “dan tien”. The remaining time was spent on meals and rest. Exercises were conducted daily according to the following schedule (Tab. 1):

Table 1. Detailed characteristic of 4 days Qigong training.

| Time of day | Thursday-Friday | |
|----------------|----------------------------|--|
| | Duration of training (min) | Kind of activity |
| 6 – 6.30 a.m. | 30 | Wake-up |
| 7–8 a.m. | 60 | Warm-up |
| 8–10 a.m. | 120 | Breakfast break |
| 10–13 a.m. | 150 | System called “5 hand movements or 6 secret wards” |
| 13 a.m.-3 p.m. | 120 | Lunch break |
| 3–6 p.m. | 150 | Static exercise: “big tree” with a break of 30 min |
| 6–8 p.m. | 120 | Dinner break |
| 8–9.30 p.m. | 90 | Meditation (small circulation 45 min, concentration at lower points 45 minutes). |
| 10–11 p.m. | 420–480 | Night break |

Genetic research. 3 ml of venous blood for genetic research was collected twice. The first and second collections were performed immediately before and after the experiment, respectively. Blood was placed in the tubes allowing separation (BD Vacutainer CPT™). The remaining PMBC were lysed using Fenzol (A & A Biotechnology, Gdynia, Poland). Further isolation of RNA was carried out by the chemical method outlined by Chomczyński and Sacchi [26]. Purity and concentration of the isolated RNA was determined by spectrophotometry (Eppendorf BioPhotometer Plus, Germany). cDNA synthesis from 2 micrograms of RNA was performed using Transcriba Kit (Roche, Department Poland) with oligo dT hexamers. For the analysis of gene expression, Real-Time PCR (Applied Biostem Step One, Lifetechnologies, Poland) was applied 3 times for the same samples using a LightCycler polymerase (Roche, Poland). The temperature-time profile of the reaction was consistent with the manufacturer's instructions. For each reaction, melt curve analysis was performed. The tubulin B gene (*TUBB*)

was used as a housekeeping gene. To amplify the genes, the following primer sequences were applied:

TUBB reverse: TCTGTCGGCTCCGCTCTGAGAT
TUBB forward: ACTCCCCTTGTCCCAAGGCTTC
HSPA1A reverse: TTCGGAGAGTTCTGGGATTGTA
HSPA1A forward: TGGACTGTTCTTCACTCTTGCC
IL6 reverse: GACATCAAGGCGCATGTGAAC
IL6 forward: TCCACGGCCTTGCTCTTGTTT
IL10 reverse: AATTCGGTACATCCTCGACGG
IL10 forward: GAATCCAGATTGGAAGCATCC
HSF1 reverse: CAGGAGCTTGAGTCCATGCA
HSF1 forward: GAGCAGTCTTGAGAACATC
NF-kB reverse: GATCCCATCCTCACAGTGTTC
NF-kB forward: TGGACTACCTGGTGCCTCTA

Statistical analysis. Descriptive statistics included the mean \pm SD for all measured variables. The normality of distribution was checked with the Shapiro-Wilk test. Gene expression data were collected and relative gene expression analysed using Excel 2010. In order to calculate the level of gene expression, the method of Schmittgen and Livak [27] was used, and data were then transformed from logarithm 2 (2^{Δ}) to a linear value. To assess the statistical significance of gene expression changes before and after exercise (WANT), a repeated measures analysis of variance (ANOVA) was used. To determine the differences in gene expression before and after the training period, a paired t-test was calculated. All calculations and graphics were performed using GraphPad Prism 6.0 (ftx.pl/program/graphpad-prism). Differences were considered to be statistically significant at the level of $p \leq 0.05$.

RESULTS

After 4 days of qigong training, a significant decrease in the expression of *CCL2* and *NF-kB* mRNA ($p=0.02$ and $p=0.002$, respectively) was observed, while *IL10* mRNA significantly increased in the same time frame ($p=0.0004$) (Fig. 1A). Genes associated with apoptosis, *HSF1* and *HSPA1A* mRNA, showed an increase in their expression. However, these changes were not significant (Fig. 1B).

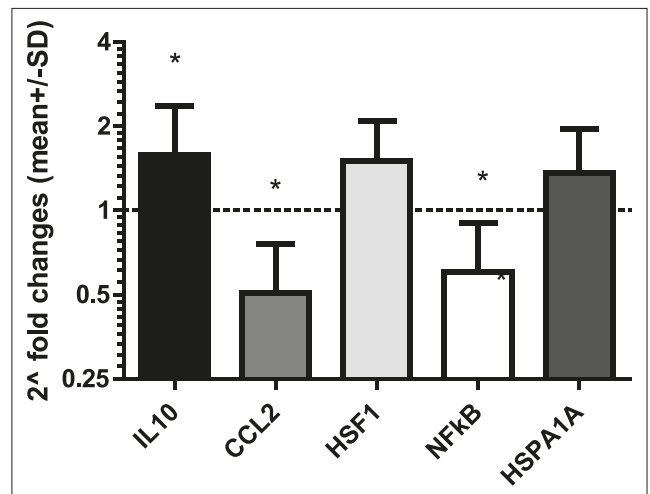


Figure 2. 2^Δfold changes in expression tested genes (*significant changes in expression)

Opposite directions of change in the expression of 2 genes associated with inflammation and heat shock proteins were observed (Fig. 2). *NF-kB* and *CCL2* mRNA significantly decreased ($2^{\Delta}0.6$ fold, $p=0.002$ for *NFkB* and $2^{\Delta}0.5$ fold $p=0.02$ for *CCL2* m-RNA). An anti-inflammatory effect of this type of training was also observed, as *IL10* mRNA was significantly higher after the training period ($2^{\Delta}1.58$ fold, $p=0.0004$).

2^{Δ} expression higher than 1 indicates an increase and less than 1 indicates a decrease. 2^{Δ} fold =1 – no changes in expression.

DISCUSSION

To the authors' knowledge, this is the first study to investigate the effects of qigong on gene expression associated with cellular stress response in PMBC in healthy women. 4 days of qigong training in this study resulted in a significant decrease in *NF-kB* and *CCL2* mRNA, as well as a significant increase in *IL10* mRNA. A slight upregulation of *HSF1* and *HSPA1A* mRNA was also detected. The level of *IL6* mRNA in PMBC was too low to be determined and was not marked. According

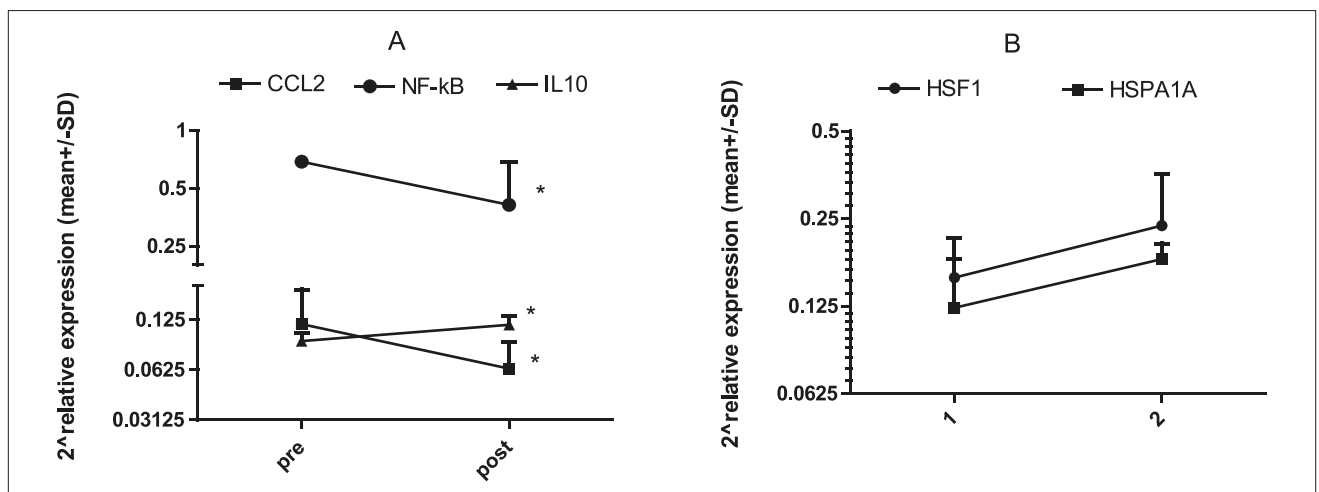


Figure 1. Changes in the relative expression (2^{Δ}) of genes associated with inflammation (1A) and heat shock protein (1B). Pre- rest value, post – value after training. (* significant changes)

to Berson [28], qigong induces a relaxation response (RR) which was confirmed by changes in the genes associated with inflammation.

Cellular stress response in women differs from the response in men. Diverse sensitivity to stress manifests itself more commonly in depressive and anxiety disorders in women [29–31]. These differences are associated with sex hormones, specifically estrogen, as evidenced by the decrease in postmenopausal symptoms [32]. Furthermore, chronic psychosocial stress has been associated with accelerated aging at the cellular level [14]. Currently, it is well known that this stress causes a decreased anti-oxidant capacity, and increased oxidative stress [33]. These changes could easily induce the expression of genes associated with stress response [34, 35]. Upregulation of *NF-κB* and proinflammatory cytokines and their receptors has been well documented. Therefore, all forms of training in which genes associated with stress response are downregulated have a positive health effect. Li et al. [18] reported that qigong can be useful in the reduction of gene expression associated with inflammation, a finding confirmed in the presented study. Additionally, the reduction of expression of *NF-κB* and enhancement of *IL10* *m*-RNA expression was similar to those obtained during physical activity, and comparable to the direction of adaptation changes in response to exercise. According to Fisher et al.³⁶ and Ziemann et al. [37], the adaptive effect of exercise decreases pro-inflammatory cytokines and increases anti-inflammatory cytokines. These results suggest that qigong is very useful in populations that have limited exercise tolerance.

There is also data available in the subject literature which shows an induction of apoptosis caused by qigong in different cells such as colorectal cancer cells [10,38] and [39]. In the presented study, upregulation of *HSF-1* mRNA and anti-apoptotic *HSPA1A* mRNA were also observed. These findings indicate that (1) the *HSF1*-dependent pathway in PMCB had been slightly activated and (2) *HSPA1A* mRNA was slightly upregulated, although less compared to those changes caused by physical effort [40].

Finally, all the indicated changes consisting in the reduction of inflammation and elevation of gene expression associated with apoptosis were noticeable in the PMBC collected from the healthy women. This suggests that qigong training is important for relaxation, not only for men but also for women, and this positive health effect can be confirmed by changes in the PMBC. Furthermore, changes in the tested gene expression are similar to those caused by physical activity indicating that qigong can be useful for people who cannot perform intense physical exercise.

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Author Disclosure Statement

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