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*Full Length Research Paper*

# Effect of “eight-trigram boxing” exercise on blood oxidative status and intestine bifidobacterium and lactobacillus count in practicers

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**The aim of this study was to evaluate the effectiveness of “Eight-trigram boxing” in reducing blood oxidative status and improving intestine benefical bacteria counts. P**articipants **were randomly assigned to “Eight-trigram boxing” exercise. After 3 months, significant increase in GSH, SOD, CAT, GSH-Px and GR activities were observed. In addition, intestine benefical bacteria bifidobacterium and lactobacillus count were markedly enhanced. It could be concluded that “Eight-trigram boxing” exercise was beneficial to practicers’ health.**

**Key words:** “Eight-trigram boxing”, antioxidant, bifidobacterium, lactobacillus.

## INTRODUCTION

Qigong has been practiced for thousands of years in China and in recent years, slowly but surely, it is spreading to the west (Yan et al., 1998). Millions of people, each year, are helping themselves get rid of all kind of diseases, ranging from minor aches and pains through to the most debilitating ones. It is becoming quite apparent that its future looks bright and that it can be beneficial to whoever practices it, regardless of sex, age, race, religion, level of education or status (Sakata et al., 2008).

The “Eight-trigram boxing”, one of Qigong, originally named Palm turning boxing, body shifting eight trigram boxing and interlocked eight trigram boxing, is characterized by changing positions of the palms and changing paces, somewhat resembling the Eight trigrams in the “Book of changes” (Pan and Feng, 2010). Through long period of exercise, the muscles are gently stretched and strengthened. The bones and ligaments become stronger. Organ stimulation occurs and blood flows. Balance and coordination are improved with an overall vitality and vigour towards life (Miao et al., 2009).

It is now accepted that free radical-mediated oxidation of biological molecules such as lipids, proteins, and DNA is involved in a variety of disorders and diseases (Sun et al., 2010; Aliyu et al., 2009). Above all, lipids are very susceptible to free radical attack and lipid peroxidation induces alterations in integrity, disturbances in fine structure, and functional loss of biomembranes.

Furthermore, lipid peroxidation mediated by free radicals proceeds by a chain mechanism, amplifying the damaging effect of free radicals and. Lipid peroxidation products are potentially cytotoxic and modify proteins and DNA (Omata et al., 2010; Hu et al., 2010; Liu, Wang and Liu, 2009). It is now accepted that lipid peroxidation is involved in the pathogenesis of various diseases, and consequently the role of free radical-scavenging antioxidants has received much attention (Kohri et al., 2009).

Bifidobacteria are considered as important probiotics and used in the food industry to relieve and treat many intestinal disorders. Bifidobacteria exert a range of beneficial health effects, including the regulation of intestinal microbial homeostasis, the inhibition of pathogens and harmful bacteria that colonize and/or infect the gut mucosa, the modulation of local and systemic immune responses, the repression of

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procarcinogenic enzymatic activities within the microbiota, the production of vitamins, and the bioconversion of a number of dietary compounds into bioactive molecules (Fu, 2009; Killer et al., 2010; Rahman et al., 2008). Lactobacillus is a genus of Gram-positive facultative anaerobic or microaerophilic bacteria. They are a major part of the lactic acid bacteria group, named as such because most of its members convert lactose and other sugars to lactic acid. They are common and usually benign. In humans they are present in the vagina and the gastrointestinal tract, where they are symbiotic and make up a small portion of the gut flora. Many species are prominent in decaying plant material. The production of lactic acid makes its environment acidic, which inhibits the growth of some harmful bacteria. Several members of the genus have had their genome sequenced (Singh et al., 2009; Pratumvinit et al., 2009; Giraffa et al., 2010).

In this study, we examined effect of “Eight-trigram boxing” exercise on blood oxidative status in practicers. Then, effect of “Eight-trigram boxing” exercise on intestine bifidobacterium and lactobacillus count were also investigated.

## PARTICIPANTS AND METHODS

### Subjects and procedures

Fifty-two subjects (38 women and 14 men aged 37.07 ± 5.16 years with a body height of 167.0 ± 8.1 cm, a body weight of 61.21 ± 6.38 kg) with no previous injuries in the year before the study were recruited. All practicers do not have “Eight-trigram boxing” exercise experience. Subjects attended a 80 min “Eight-trigram boxing” exercise 3 times per day for 5 months, located at the community center. During practice, blood was taken from all subjects per month. Feces were also collected from all subjects per month.

### Biochemical analysis

TC, TG, LDL-c and HDL-c levels were determined using spectrophotometer and standard commercial kits.
GSH level was determined by DTNB method (Nannelli et al., 2007). MDA level was examined with TBA fluoremetry (Hepgül et al., 2010). And the total SOD activity was determined by pyrogallol-NBT colorimetry (Cigremis et al, 2009). The CAT activity in liver extracts was measured spectrophotometrically at 240 nm by calculating the rate of degradation of $H_2O_2$, the substrate of the enzyme (Żwirska-Korczala et al., 2004). Glutathione peroxidase (GSH-Px, EC 1.6.4.2) activity was measured by the method of Kocer et al. (2006). Glutathione reductase (GR) activity was measured using the procedure described by Błaszczyk et al. (2008).

### Counting of bifidobacteria and lactobacillus in feces

Feces of subjects were analyzed for the number of total bifidobacteria and lactobacillus using the procedure described by Kawase et al. (1982).

### Statistical analysis

Statistical analysis was performed using the SPSS-PC 11.0 software (SPSS Inc., Chicago, Illinois, USA). One-way ANOVA and LSD test were performed in order to determine whether there was any significant difference during different exercise time.

## RESULTS

### Effect of “eight-trigram boxing” on MDA and GSH levels

The results of the changes of MDA and GSH levels in practicers’ blood are represented in Table 1. As can be observed, there was a decrease in MDA level and increase in GSH level, respectively. In both cases, the effect was statistically significant ($P < 0.05$; $P < 0.01$). These effects were strengthened with increasing exercise time.

### Effect of “eight-trigram boxing” on TC, TG, LDL-c and HDL-c levels

The results of the changes of TC, TG, LDL-c and HDL-c levels in practicers are represented in Table 2. As can be observed, there was a decrease in TC, TG, and LDL-c levels. On the contrary, HDL-c level increased significantly ($P < 0.05$; $P < 0.01$). In both cases, the effect was statistically significant ($P < 0.05$; $P < 0.01$). These effects were shown to develop progressively over exercise time.

### Effect of “eight-trigram boxing” on SOD, CAT, GSH-Px and GR activities

As demonstrated in Table 3, SOD, CAT, GSH-Px and GR activities in practicers were significantly ($P < 0.05$; $P < 0.01$) increased. The effect were significantly ($P < 0.05$; $P < 0.01$) increased in a time-dependent manner. Namely, the effect increased with increasing exercise time.

### Effect of “eight-trigram boxing” on intestine bifidobacterium and lactobacillus count

Table 4 showed the role of “Eight-trigram boxing” in modulating intestine bifidobacterium and lactobacillus count during exercise. Highly significant enhancement ($P < 0.05$; $P < 0.01$) in the count of intestine bifidobacterium and lactobacillus were obtained during exercise. Moreover, with increasing exercise time, intestine bifidobacterium and lactobacillus count accordingly increased.

**Table 1.** Effect of "Eight-Trigram Boxing" on MDA and GSH levels.

| | 0 (month) | 1 (month) | 2 (month) | 3 (month) | 4 (month) | 5 (month) |
|---|---|---|---|---|---|---|
| MDA (mmol/mg) | 12.38±1.07 | 12.15±1.13 | 9.02±1.11[a] | 7.47±0.63[b] | 7.05±0.66[b] | 6.53±0.54[b] |
| GSH (μmol /g) | 44.25±2.93 | 49.68±2.08[a] | 58.04±3.71[b] | 63.75±5.07[b] | 77.12±5.39[b] | 80.62±7.31[b] |

[a] $P < 0.05$, [b] $P < 0.01$, compared with control (0 month).

**Table 2.** Effect of "Eight-Trigram Boxing" on TC, TG, LDL-c and HDL-c levels.

| | 0 (month) | 1 (month) | 2 (month) | 3 (month) | 4 (month) | 5 (month) |
|---|---|---|---|---|---|---|
| TC (mmol $L^{-1}$) | 1.73±0.18 | 1.68±0.11 | 1.54±0.08[b] | 1.48±0.11[b] | 1.32±0.06[b] | 1.28±0.10[b] |
| TG (mmol $L^{-1}$) | 0.39±0.04 | 0.34±0.03 | 0.31±0.02[b] | 0.29±0.03[b] | 0.29±0.02[b] | 0.27±0.02[b] |
| LDL-c (mmol $L^{-1}$) | 0.42±0.04 | 0.41±0.03 | 0.38±0.04[a] | 0.34±0.03[b] | 0.31±0.04[b] | 0.29±0.03[b] |
| HDL-c (mmol $L^{-1}$) | 1.31±0.21 | 1.46±0.14[a] | 1.57±0.21[b] | 1.63±0.15[b] | 1.82±0.16[b] | 2.06±0.15[b] |

[a] $P<0.05$, [b] $P<0.01$, compared with control (0 month).

**Table 3.** Effect of "Eight-Trigram Boxing" on SOD, CAT, GSH-Px and GR activities.

| | 0 (month) | 1 (month) | 2 (month) | 3 (month) | 4 (month) | 5 (month) |
|---|---|---|---|---|---|---|
| SOD(U/mg) | 79.09±6.09 | 89.33±6.77[b] | 98.09±6.92[b] | 132.11±11.65[b] | 146.08±12.35[b] | 153.07±11.09[b] |
| CAT(U/mg) | 9.56±0.87 | 12.07±0.69[a] | 15.25±1.63[b] | 17.07±1.12[b] | 27.09±2.83[b] | 28.14±2.04[b] |
| GSH-Px(U/mg) | 18.95±1.43 | 24.75±2.09[b] | 30.09±1.79[b] | 32.11±2.48[b] | 34.09±2.16[b] | 35.01±1.95[b] |
| GR(U/mg) | 14.96±1.08 | 18.09±1.67[a] | 23.75±1.85[b] | 25.09±2.84[b] | 26.11±1.75[b] | 28.09±2.94[b] |

[a] $P<0.05$, [b] $P<0.01$, compared with control (0 month).

**Table 4.** Effect of "Eight-Trigram Boxing" on intestine bifidobacterium and lactobacillus count (log CFU /g, $x \pm s$, $n = 5$).

| | 0 (month) | 1 (month) | 2 (month) | 3 (month) | 4 (month) | 5 (month) |
|---|---|---|---|---|---|---|
| bifidobacterium | 7.45±0.31 | 8.24±0.75[a] | 8.89±0.57[b] | 9.23±0.77[b] | 9.64±0.83[b] | 10.42±0.65[b] |
| lactobacillus | 11.57±0.93 | 12.57±0.58[a] | 13.08±1.08[b] | 13.65±1.32[b] | 14.01±2.09[b] | 15.19±1.94[b] |

[a] $P<0.05$, [b] $P<0.01$, compared with control (0 month).

## DISCUSSION

Age, diet, and strenuous exercise could all causes oxidative stress, resulting in lipid peroxidation and DNA damage (Błaszczyk et al., 2010; Qiao et al., 2009).
Oxidative injury and inflammatory response involving oxygen free radicals have been implicated to be of vital importance in healthy people and patients, which sometimes lead to higher morbidity (Jia et al, 2009; Fan et al, 2009).

This work presents the results from a controlled experiment where the effect of "Eight-trigram boxing" exercise on changes in plasma lipid peroxidation, capacity antioxidant and intestine bifidobacterium and lactobacillus count in practice. In these trained subjects, a significant increase in GSH levels, SOD, CAT, GSH-Px and GR activities was found in relation to exercise. In addition, a decrease in TC, TG, LDL-c levels and increase in HDL-c were detected in practicers. Moreover, these benefical effects were further improved with increasing exercise time. These suggest that "Eight-trigram boxing" exercise enhanced antioxidant status and reduced oxidative damage in practicers' body.

Probiotic bacteria favorably alter the intestinal microflora balance, inhibit the growth of harmful bacteria, promote good digestion, boost immune function, and increase resistance to infection (Smirnov et al., 1993). People with flourishing intestinal colonies of beneficial bacteria are better equipped to fight the growth of disease-causing bacteria (Mel'nikova et al., 1993; De Simone et al., 1993). Lactobacilli and bifidobacteria maintain a healthy balance of intestinal flora by producing organic compounds—such as lactic acid, hydrogen peroxide, and acetic acid—that increase the acidity of the intestine and inhibit the reproduction of many harmful bacteria (Veldman et al., 1992; Kawase, 1982). Probiotic bacteria also produce substances called bacteriocins, which act as natural antibiotics to kill undesirable microorganisms (Rasic, 1983). Our work showed that “Eight-trigram boxing” exercise could increase intestine bifidobacterium and lactobacillus count.

## Conclusion

Cumulative ROS generated in body could induce oxidative injury. “Eight-Trigram Boxing” exercise could activate antioxidant enzymes activities and reduced lipid peroxidation level. In additionm, “Eight-trigram boxing” exercise could still increased intestine bifidobacterium and lactobacillus count. Furthermore, the effect became better with prolonging exercise period. These results suggest that “Eight-trigram boxing” is useful for practicer’s health.

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