The authors investigated whether thoracic lymphatic pumping (TLP) after FluShield vaccination enhanced the production of anti-influenza immunoglobulins in elderly individuals, who are at particular risk for influenza. Osteopathic students and non–TLP-treated elderly subjects served as controls. Serum antibody titers were quantified with enzyme-linked immunosorbent assay, and hemagglutination inhibition assay, both of which generated comparable results. While approximately 70% of the younger controls had increased anti-influenza immunoglobulin production on vaccination, only 30% to 35% of the aged population had increased antibody production. There was no significant enhancement in anti-influenza immunoglobulin production in the TLP-treated subjects.

The authors’ findings suggest that TLP in conjunction with influenza vaccination does not enhance immunization against influenza in otherwise healthy and active populations. However, such techniques may be of value when applied in conjunction with vaccination to nonambulatory patients or on actual influenza exposure of at-risk individuals.

(Key words: osteopathic manipulative treatment, thoracic lymphatic pumping, influenza vaccination, immune response)

A number of historical references indicated that osteopathic modes of therapy, especially methods that involved lymphatic pumping, were efficacious for enhancing immune response. Whiting reported that osteopathic manipulation of the liver and spleen increased the phagocytic index of blood cells, and Smith reported extraordinary rates of increased antibody production. There was no significant enhancement in anti-influenza immunoglobulin production in the TLP-treated subjects.

The authors’ findings suggest that TLP in conjunction with influenza vaccination does not enhance immunization against influenza in otherwise healthy and active populations. However, such techniques may be of value when applied in conjunction with vaccination to nonambulatory patients or on actual influenza exposure of at-risk individuals.

(Key words: osteopathic manipulative treatment, thoracic lymphatic pumping, influenza vaccination, immune response)
Subjects
Thirty-six young adults and 61 elderly adults volunteered for this study. The younger subjects were first- and second-year osteopathic medical students, while the older subjects were recruited from local facilities that housed or catered to elderly populations. All subjects completed an informed consent form, and the experimental protocols were performed as approved by the Institutional Review Board of Des Moines University Osteopathic Medical Center. After completing screening questionnaires, subjects were given physical examinations under the supervision of a primary care physician (M.C.). Individuals with contraindications to influenza vaccine were excluded from the study, and the subjects selected for this investigation were found to be healthy and physically active. Subjects in each age group were randomly and equally divided into either the TLP or control group. Average age (±SD) for the young group was 26.9 ± 1.5 years for the control subjects and 27.2 ± 4.3 years for the TLP subjects, while average age for the elderly subjects was 69.2 ± 5.4 years for the control subjects and 69.1 ± 5.1 for the TLP subjects.

Thoracic lymphatic pumping
Thoracic lymphatic pumping was performed by a physician certified in osteopathic manipulative medicine (D.B.) or one of his trained fellows of osteopathic manipulative medicine. To perform TLP, the practitioner places his or her hands below the clavicles bilaterally, with fingers fanned out across the front and sides of the chest. The practitioner “pumps” the hands in a quick, rhythmic fashion, fifty pumps per minute for 5 minutes. In this protocol, the treatment groups received a daily TLP treatment for 4 days postvaccination.

Obtaining and processing blood samples
A baseline blood sample (10 mL) was obtained, and the FluShield vaccine was administered. For the treatment groups, TLP was performed once per day on the following four days. Another blood sample was obtained during the fourth week (average 30 days) postvaccination.

Blood samples were allowed to clot, then centrifuged within 2 hours of venipuncture. Serum was immediately removed and refrigerated at 4°C. Within 24 hours, each serum sample was divided into four aliquots and stored at −25°C until analysis. The individuals performing the serum analyses (T.B., K.H., and J.E.) were blinded with regard to sources (that is, donor age, TLP status) of the samples.

Serum analyses
The serum samples were analyzed using both hemagglutination inhibition assay (HIA) and enzyme-linked immunosorbent assay (ELISA). The HIAs were performed as described by protocols provided by Wyeth-Ayerst and Bio-Whittaker. Individual viral components comprising the trivalent 1996/1997 FluShield were provided by Wyeth-Ayerst. After preliminary HIA studies using these components, we chose the Nanchang antigens for our HIAs and ELISAs. The newly found Nanchang virus was predicted to be the major infective viral strain for 1996 and 1997, and the use of these components decreased the background interference in our assays.

Serum samples were digested overnight at 37°C with receptor-destroying enzyme (RDE; Bio-Whittaker). After RDE digestion, the mixture was heated for 30 minutes at 56°C, the serum was diluted 1/10 with isotonic phosphate-buffered saline (PBS), immediately cooled to 4°C, and then promptly used for assay.

Figure 1. Hemagglutination inhibition assay (HIA) response across age group and thoracic lymphatic pumping (TLP) condition. Younger subjects had a significantly (P < .01) higher percentage of positive responders than did the elderly subjects. There were no significant (P < .05) differences between TLP and control conditions. The numbers above each bar represent the number of subjects in that particular group.
Fresh chicken red blood cells (RBC; Hy-Vac) were washed several times in PBS, then diluted to 1% RBC (v/v) in PBS. Pre- and postvaccination serum samples were serially diluted in 96 well HIA microtiter plates. Fifty microliters of the Nanchang antigen (diluted 1/8000) and 50 μL of 1% RBC were added to each well. The solutions were gently mixed for 1 minute, and then the plates were incubated overnight at 4°C. The next morning, the plates were read and scored independently by three investigators. Plates were then reviewed collectively, the HIA readings were discussed, and the data were pooled.

All samples were run in duplicate on separate HIA plates, and each serum sample was assayed three times, totaling six data points per sample. A positive HIA response was an average one well difference (six wells total) between the pre- and postvaccination serum samples. This value approximates a twofold increase in anti-Nanchang immunoglobulin concentration.

For the ELISA analyses, 96 well ELISA microplates were prepared by adding 100 μL of Nanchang antigen (diluted 1/100 in NaHCO₃, pH 9.6) to each well of the plate. The plates were incubated for 2 hours at ambient temperature. The antigen solution was removed, and the wells were blocked twice with 400 μL of 1% bovine serum albumin (BSA) in PBS for 30 minutes at ambient temperature. On removal of the BSA, the plates can be used immediately for ELISA or air-dried and stored in sealed containers at 4°C for up to 2 weeks.

RDE-digested serum was diluted with 0.1% BSA in PBS to give final dilution factors of 1/8000 and 1/16,000. Two hundred-microliter aliquots of each dilution were added to duplicate plates, and then incubated for 1 hour at ambient temperature. Next, the plates were washed three times with isotonic Tween/tris-buffered saline (TTBS) via an ELISA microplate washer (BIO-TEK). Fifty microliters of peroxidase-conjugated goat antihuman IgA, IgG, IgM (Pierce) diluted 1/10,000 in TTBS was added to each well, then incubated 1 hour at ambient temperature. The microtiter plates were then washed three times with TTBS. One hundred microliters of 3,3′,5,5′-tetramethylbenzidine peroxidase color substrate (Pierce) was added to each well and allowed to react for exactly 2.5 minutes per well. The reaction was stopped by addition of 1 N H₂SO₄. The color was quantitated at 450 nm with a computer-controlled ELISA microplate reader (BIO-TEK). The ELISA data were imported into a spreadsheet and statistically analyzed.

**Statistical analyses**

The ELISA data were analyzed with a two-factor (age group × TLP condition) mixed analysis of variance, and the HIA data (dependent variable = positive vs. negative response) were analyzed with Chi-square tests. All statistical tests were performed at the .05 alpha level.

**Results**

Of the 18 subjects in the young control group, 9 returned to donate the postimmunization blood sample, while 16 of 18 of the TLP-treated subjects returned to donate the 4-week (average 30 days) sample. Of the 61 subjects in the older group, 26 of 29 untreated controls and 28 of 32 TLP-treated subjects returned to donate the postimmunization blood sample.

Figure 1 illustrates the percentages and numbers of positive and negative responders as determined by HIA analysis, within control and TLP-treated subjects. An omnibus Chi-square analysis (includes both age groups and TLP treatment groups) was not significant, nor was a comparison between TLP- and non–TLP-treated subjects (data collapsed across age group). These results showed no significant effect for TLP treatment. However, there was a significant effect for age group (P < .01; data collapsed across TLP condition), indicating that younger subjects had a significantly higher percentage of responders than the older subjects. In the younger group, the percentage of HIA positive responders was essentially identical for the control subjects (67%) and for the TLP-treated subjects (69%). The closeness of these percentages is remarkable considering the small sampling for the nontreated control group. Figure 1 also shows that the
younger subjects had twice the percentage of positive responders, with almost 70% responding to vaccination versus 33% for the older subjects. Within the older group, 8 (31%) of 26 subjects in the control condition had a positive response, while 10 (36%) of 28 of the TLP-treated subjects showed a positive response to vaccination.

The HIA data can also be used to indicate the strength of the immune response for each subject, as each well difference between prevaccination and postvaccination samples indicates an approximate doubling of anti-influenza antibody concentration. Thus, larger differences indicate greater production of anti-influenza antibodies. Figure 2 shows the distribution of subjects according to the strength of their immune response.

With the older subjects, 13 (72%) of 18 of the positive responders had a twofold or fourfold antibody increase on vaccination, while with the younger subjects, 13 (72%) of 18 of the positive responders had an eightfold or greater antibody increase on vaccination. Therefore, the HIA results show that the younger subjects had a greater percentage of positive responders and a more robust immune response. TLP did not appear to change the quantity or quality of the immune response.

The ELISA analysis is summarized in Figure 3. Neither the age group by TLP interaction nor the main effect for TLP condition was significant. Like the HIA data, these results indicate that the TLP treatments did not increase the antibody response to the vaccination in either age group. In accordance with the HIA data, the main effect for age group was significant (F = .0001). The young subjects exhibited a larger average absorbance difference (ΔAb450) than the older subjects (0.36 ± 0.20 vs. 0.17 ± 0.19), irrespective of TLP treatment, and the ELISA absorbance differences between the age-matched experimental groups were essentially identical.

**Comments**

We suggest that this study establishes a benchmark for comparing healthy individuals to individuals who are good candidates for lymphatic treatment. A key strategy in our experimental design was to test healthy, active, and ambulatory individuals. These protocols used two distinct and reproducible methods, HIA and ELISA, to determine antibody production in response to influenza vaccination. In this experimental protocol, the individuals performing the antibody analyses were blinded until the assay series were completed.

Our studies show that a higher percentage of young adults responded positively to the vaccine than older individuals. This observation has been reported by Bernstein. While approximately 70% of the young controls had increased antibody production on vaccination, only about 30% to 35% of the older population had a significant response. These data also demonstrate that among young and elderly positive responders, young individuals generated higher levels of protective antibodies. Thus, the aged subjects had a decreased immune response, both quantitatively and qualitatively.

Our results suggest that TLP had little therapeutic advantage for enhancing the production of antibodies against influenza antigens in healthy, active, ambulatory individuals—young or aged. These data illustrate the importance of an active lifestyle, as the muscle contractions involved in normal movement and respiration contribute significantly to lymphatic circulation.

However, lymphatic pumping may enhance the immune response toward other vaccines. Results of the study by Jackson and coworkers indicated that immune pumping enhanced antibody production on vaccination with the hepatitis B vaccine. One factor that should be considered when comparing our results with theirs is the nature of the vaccination protocol. Influenza immunization requires one inoculation with no booster, while hepatitis B vaccination involves an initial vaccination and two subsequent boosters. The lymphatic methods used in each study and the timing and frequency of the treatments also should be considered. The study by Jackson and others used six sessions of splenic and thoracic pumping over 2 weeks after each inoculation, while we followed one inoculation with four TLP sessions over 4 days. Considering these differences, it is
interesting that their data are in full accord with ours over the course of our protocol (30 days), as they did not see pump-enhanced antibody responses until 7 weeks into the study and 2 weeks after the first booster injection.

Other reports indicate that lymphatic pumping techniques enhanced immune function or improved clinical outcomes in patients with respiratory disease and lymphatic stasis.4,6,7,17,18 Thus, TLP in conjunction with other osteopathic modes of therapy could be especially efficacious on vaccination of sedentary or bedridden populations. Considering the booster effect noted previously,9 influenza-vaccinated elderly individuals might benefit from lymphatic pumping on subsequent exposure to the influenza viruses.

An aspect of the immune response that our study did not address directly is the concomitant cell-mediated events that occur on vaccination. The preliminary studies of Measel and Kafity10 and Mesina and others11 were important steps in this direction. Both groups demonstrated that lymphatic pumping altered immune blood cells. However, their studies were not antigen-specific. A sophisticated determination of antigen-specific generation memory T cells and B cells would add to our understanding of lymphatic pumping and immune enhancement. We have the scientific tools to determine the immune mechanisms and appropriate clinical roles of lymphatic pumping. We now need to apply insightful experimental protocols to the appropriate patient populations.

References
2. Smith RK. One hundred thousand cases of influenza with a death rate of one-fortieth of that officially reported under conventional medical treatment. JAOA 1920;19:172-175.