Signals Consistent with Microbubbles Detected in Legs of Normal Human Subjects After Exercise

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Introduction

- Bubbles can form in humans undergoing decompression.
- A much greater pressure difference is needed to form bubbles in blood outside of the body than is needed to form bubbles inside of the body.
- Naturally occurring micronuclei may explain this discrepancy by providing nucleation sites for bubbles to form.
- Direct measurement of tissue micronuclei in humans has not previously been possible due to their assumed small size and lack of motion.
- A number of studies have shown that exercise before and during decompression can increase risk for decompression sickness (DCS).
- The increased risk may be due to micronuclei produced during exercise.
- The goal of this study was to detect micronuclei in normal humans after exercise.

Dual Frequency Ultrasound

- Bubbles behave nonlinearly when resonating.
- Bubbles driven at resonance will produce mixing (sum and difference) signals if simultaneously driven at a second, higher frequency.
- Dual-Frequency Ultrasound (DFU) involves "pumping" bubbles at their resonant frequency and "imaging" the bubbles using a second frequency.
- DFU can detect smaller bubbles than B-Mode ultrasound and also can be used to measure stationary bubbles.

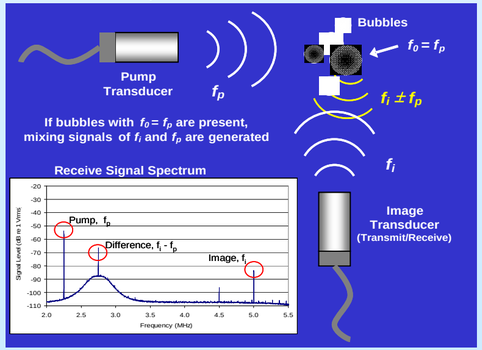

Figure 1. Dual-frequency ultrasound looks for the nonlinear mixing of two different ultrasound frequencies to detect and size resonating bubbles.

Test Protocol

- Six normal human subjects rested for 120 minutes, then rode a stationary bicycle for 30 minutes at 80% of age-predicted maximal heart rate.
- Six DFU measurements were taken at multiple sites starting 60 minutes before exercise. Measurements continued for 60 minutes after exercise (Figure 2).
- Measurements were made at 11 locations over the hamstring, quadriceps, and gastrocnemius muscles of the left leg (Figure 3).

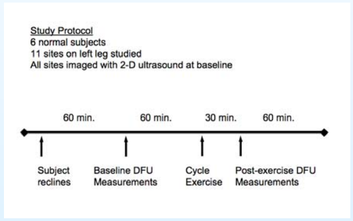

Figure 2. Protocol followed during the human subject tests.

Figure 3. Measurement locations on left thigh.

Data Analysis

- Repeated-measures ANOVA performed on all data from each site at all time points.
- A measurement was considered "positive" if significantly ($p<0.01$) greater than any baseline measurement at the same site on the same subject (i.e., each subject and site provided its own baseline).
- Overall number of sites with positive measurements evaluated using a Chi-square test.

Results

- 44 of 66 sites showed positive signals after exercise.
- 7 sites showed positive baseline signals.
- A typical time course involved large signals immediately after exercise, which decayed during the recovery period (Figure 4).
- In some cases, a delayed response was observed (Figure 5).
- Difference between baseline and post-exercise measurements highly significant ($p<0.0001$) (see Figure 6).
- Figure 7 shows the fraction of all signals measured above a particular level for baseline, all post-exercise, and positive post-exercise populations. This plot clearly shows the difference between these populations.
- Figure 7 also suggests appropriate threshold detection levels for future DFU implementations.

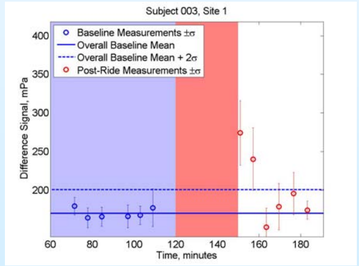

Figure 4. Typical time course from study.

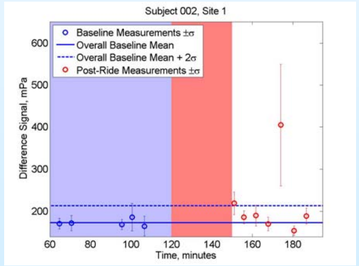

Figure 5. Time course exhibiting delayed response.

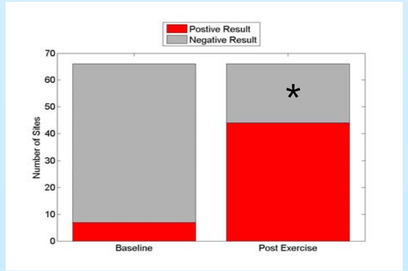

Figure 6. After exercise, 44 of 66 sites had positive signals, while only 7 sites had a positive baseline measurement.

Discussion

- Signals consistent with microbubbles were detected in the legs of normal human subjects after exercise.
- This work demonstrates the first *direct* measurement of exercise-induced tissue microbubbles.
- Measurements suggest that exercise-induced microbubbles disappear quickly following exercise.
- Positive baseline signals could be naturally-occurring, stable microbubbles.

Future Work

- The ability to measure tissue micronuclei could provide several novel capabilities, such as:
  - -New capability to optimize and evaluate decompression schedules.
  - -New capability to evaluate non-compressive DCS treatments.
  - -Could potentially be used to diagnose DCS.
  - -Could be used to evaluate bubble growth models.
  - -May be used to evaluate pre-breathe strategies.

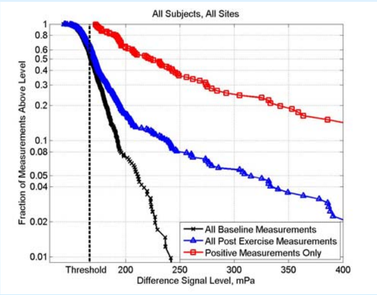

Figure 7. Plot showing the fraction of measurements above a given signal level for various populations.

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