Short communication

Speed of sound in muscle for use in sonomicrometry

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ABSTRACT

Converting ultrasound transit time into a measure of distance when using sonomicrometry requires that the speed of sound be known. A number of different values for the speed of sound in muscle have been assumed in studies of skeletal and cardiac muscle, and in some cases the effect of temperature has been ignored. The speed of ultrasound with frequencies greater than 1 MHz in skeletal and cardiac muscle is briefly reviewed, including the effects of temperature and contractile state. A simplified equation for the speed of sound in pure water is presented for the temperature range from 0–50 °C. This equation can be used when calibrating sonomicrometer transducers in water. The data available indicate that the speed of sound in both cardiac and skeletal muscle can be approximated by multiplying the speed of sound in pure water at the measurement temperature by 1.045. Differences in the speed of sound in the longitudinal and transverse directions and changes with contractile state appear to be small and in most cases can probably be safely ignored. The normal variation in muscle composition does not greatly affect the speed of ultrasound in muscle, but investigators placing sonomicrometer transducers near tendons should be conscious of the much greater speed of sound in tendon and variation with loading.

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1. Introduction

Sonomicrometry is a common tool in studying the function of skeletal as well as cardiac muscle. With this technique the transit time of an ultrasound pulse between two piezoelectric transducers is used to estimate distance. To convert the transit time into a measure of distance requires that the speed of sound be known. Because the measured distance is simply the speed of sound multiplied by the transit time, the percentage errors in the speed of sound and the distance will be the same. A number of different values for the speed of sound in muscle have been assumed in studies of skeletal and cardiac muscle, and in some cases the effect of temperature has been ignored. For example, in a number of studies a general value for speed of sound in mammalian tissue of 1540 m s^{-1} has been assumed, which is probably 4% smaller than the appropriate value for skeletal muscle at a typical mammalian body temperature of 37 °C. In some cases the speed of sound does not affect the conclusions of studies of *in vivo* muscle function because distance values are converted to fractional length change or strain. Assuming that other sources of error that create offsets in length are eliminated, e.g., triggering errors and the lens effect, the relative change in length is calculated accurately despite any error in calculating the absolute length. However, in some cases the absolute distance does matter, e.g., in measuring cardiac dimensions (Gaynor et al., 1994), or calculating the volume of fluid expelled during jet propulsion (Marsh et al., 1992). Knowing the appropriate speed of sound for calibration conditions in water compared with the conditions *in vivo* is also important. This review aims to clarify the state of our knowledge of the speed of ultrasound in skeletal and cardiac muscle, including the effects of temperature and contractile state. I consider only studies of the speed of sound with frequencies above 1 MHz that are relevant to the ultrasound pulses used in sonomicrometry. Sound waves at frequencies in the low kHz range behave differently in muscle, including having a marked frequency dependence of speed (Truong, 1974).

2. Speed of Sound in Pure Water

The speed of sound in pure water at different temperatures is known very accurately (Del Grosso and Mader, 1972; Bilaniuk and Wong, 1993, 1996). The effects of salinity and pressure on this speed have also been quantified (Mackenzie, 1981; Belogol'skii et al., 1999). (For equations and interactive calculations see: http://www.npl.co.uk/acoustics/acoustic-technical-guides.) Therefore, the known values for pure water have often been used as standards in measuring the speed of sound in tissues. Using data from

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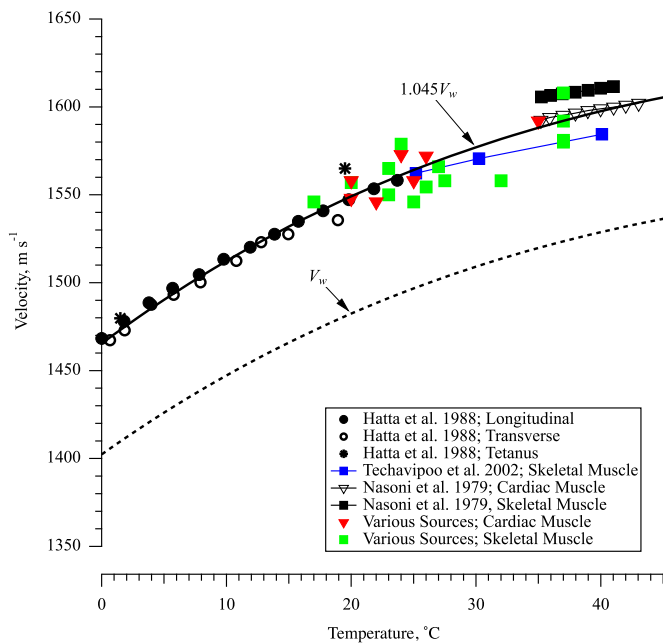
Fig. 1. Speed of sound in skeletal and cardiac muscles at various temperatures. Also shown are curves for the speed of sound in pure water (V_w) based on Eq. (1) and for $1.045V_w$. The values indicated as from "Various Sources" include measurements on muscles from rats, frogs, rabbits, sheep, cattle, dogs, pigs, and humans and were obtained from the following: Bhagat et al., 1977; Chivers and Parry, 1978; Goss et al., 1978; Bowen et al., 1979; Mol and Breddels, 1982; Levinson, 1987; Park et al., 1994; Topp and O'Brien, 2000; Marutyan et al., 2006.

Del Grosso and Mader (1972), Bilaniuk and Wong (1993, 1996) described the speed of sound in water over the temperature range from 0 to 95 °C with a fifth order polynomial with constants to 9 significant figures. To produce a simpler equation for biologically relevant temperatures, I fitted a third order polynomial using the same data, but restricted to temperatures from 0 to 50 °C. The resulting equation has a maximum deviation from the equation of Bilaniuk and Wong (1996) of 0.09 m s^{-1} over this temperature range. The simplified equation is:

$$V_w = 1402.4 + 5.0167\,T - 0.05505T^2 + 0.00021588\,T^3 \qquad (1)$$

where, V_w is the speed of sound in pure water in m s^{-1} and T is temperature in °C. This equation can be used when sonomicrometer transducers are calibrated in water.

3. Speed of Sound in Muscle

One of the more useful sets of measurements for researchers interested in the contractile function of skeletal muscle is that of Hatta et al. (1988), whose goal was to measure the change in stiffness of frog skeletal muscle during various conditions. This work is particularly valuable because they used living muscles at known sarcomere lengths and made measurements of both longitudinal and transverse speeds over a broad temperature range (Fig. 1). The speed in resting muscle varied with temperature as expected from the thermal variation of speed in water. The longitudinal speed in skeletal muscle was approximately 4.5% greater than the speed in water over the temperature range from 0 to 23 °C. Speed in the transverse direction in resting muscle was approximately 0.8% less than that in the longitudinal direction (Fig. 1). This small difference depending on the direction of measurement is similar to the differences (0.6–0.8%) found by Mol and Breddels (1982) and Marutyan et al. (2006) in excised blocks of skeletal and cardiac muscle. The velocity of sound in resting

muscle was not influenced by passive lengthening that increased force (Hatta et al., 1988).

Numerous other estimates of the speed of sound at different temperatures in non-contracting skeletal and cardiac muscle exist and the variability among these values is considerable (Fig. 1). These values extend the range of measurement temperatures through the highest values encountered in mammalian and avian studies *in vivo*. Much of the variability likely stems from different techniques of specimen preparation and measurement. Therefore, I have, where appropriate, averaged measurements taken by the same authors at the same temperatures. Based on the available data, the speeds appear similar in skeletal and cardiac muscle (Fig. 1). Two studies (Nasoni et al., 1979; Techavipoo et al., 2002) examined thermal effects at higher temperatures than those used by Hatta et al. (1988). Both of these studies appear to show lower thermal sensitivities than that found for water (Fig. 1). However, in both studies the possibility exists that the temperature in the sample was not uniform due to thermal lag. Considering all of the data, the speed of sound in muscle across the entire temperature range is reasonably approximated by a curve equal to 1.045 times the speed of sound in water (Fig. 1). In the data summarized in Fig. 1, the deviations from this curve amount to at most a 1.5% error.

Available studies provide contradictory data on the effect of contractile activity on the speed of sound in muscle, and leave open the question of any practical impact on studies of muscle function with sonomicrometry. Hatta et al. (1988) found an increase in the longitudinal speed of sound during maximal isometric tetani in isolated frog muscle. The increase was approximately 0.5 and 1.3% at 1.5 and 19.5 °C, respectively (Fig. 1). Conversely, Caputi et al. (1992) using an *in situ* preparation in cats found no change in longitudinal speed. Because numerous studies using mechanical perturbations have documented an increase in longitudinal stiffness resulting from crossbridge formation, the finding of Hatta et al. (1988) of an increase in the longitudinal speed of sound during an isometric tetanus appears to have a mechanistic explanation. However, the estimates of stiffness based on the speed of sound in the study of Hatta et al. (1988) differ in some respects from other information on stiffness in muscle. First, the effect of temperature on stiffness during contraction in frog muscle measured with mechanical perturbations (Bressler, 1981) is much smaller than that suggested by the data of Hatta et al. (1988). Second, Hatta et al. (1988) measured a smaller increase in the longitudinal speed of sound at 19.5 °C in muscles in rigor (0.8%) than that found during contraction (1.3%). This finding is surprising if the changes in speed are due to changes in stiffness, because other studies demonstrate that muscle fibers in rigor are much stiffer than during active contraction because more crossbridges are attached in rigor (Linari et al., 2007). The inconsistencies in interpreting the data of Hatta et al. (1988) in terms of stiffness raise the distinct possibility that some other factor contributed to the apparent increase in longitudinal speed with maximal contraction. For transverse speed, although two studies (Hatta et al., 1988; Bhagat et al., 1976) have suggested a small decrease (0.6–1.3%) in the speed of sound during maximal isometric tetani, three others (Mol and Breddels, 1982; Levinson, 1987; Caputi et al., 1992) have found no change. No convincing mechanistic explanation exists that would explain a decrease in transverse speed during contraction. If anything, one might predict an increase in velocity because crossbridge attachment appears to cause an increase in radial stiffness at least at the single fiber level (Brenner and Yu, 1991). Overall, the evidence for changes in the speed of ultrasound in contracting muscle is limited and any effect during *in vivo* recording involving submaximal contractions would likely be undetectable. Until more data are collected on resting and active muscle, perhaps some caution is