The role of glycogen and phosphate in ultrasonic attenuation of liver

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The excess ultrasonic attenuation caused by adding glycogen and inorganic phosphate to liver homogenates has been studied to determine the underlying differences between attenuation coefficients of normal and diffusely diseased livers. Results show that glycogen has a higher than average specific absorption coefficient compared to other large molecular weight biomolecules. Since the glycogen content of liver can vary from 1%–10% of wet weight, this compound may have a major time-varying effect on the liver ultrasonic attenuation coefficient, even in normal subjects. In contrast, the excess attenuation of liver homogenate resulting from addition of inorganic phosphate was not significant at presumed physiological levels. The implications of these findings are discussed relative to tissue characterization efforts based on *in vivo* measurements of ultrasonic attenuation coefficients of liver.

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INTRODUCTION

The ultrasonic attenuation coefficient of tissues has potential as a useful diagnostic or tissue characterization parameter. For example, the magnitude and frequency dependence of attenuation in livers have been shown to be affected by carbon tetrachloride toxicity[1] and by diffuse liver diseases.[2,3] A number of investigations have addressed the underlying mechanisms of attenuation in tissues. It is clear that multiple relaxation mechanisms at the macromolecular level are responsible for absorption (conversion of wave energy to heat) in tissues,[4,5] and, furthermore, that absorption dominates attenuation in liver and brain tissues.[6–9] However, the specifics of how liver attenuation might change with disease remain unclear. The roles of fat[1,10,11] and collagen [12,13] are implicated as contributors to increased tissue attenuation, but the correlation of any single histological parameter, such as increased fat content, with increased attenuation generally yields a wide scatter in results.[3,10,11] Thus other factors are likely to play a major role in contributing to the liver attenuation and absorption coefficients. Necrosis and swelling with low attenuating fluids are factors that probably act to lower the attenuation of an intoxicated liver.[1] Other theoretical and empirical results have led us to consider the roles of glycogen and inorganic phosphate as contributors to liver attenuation.

Glycogen is manufactured in the liver from available glucose and is stored in hepatocytes as high molecular weight ($>10^6$) molecules.[14,15] The stored glycogen represents available energy reserves for the liver and body, and can vary from less than 1% to approximately 10% of the liver wet weight, depending on "fasting or feasting" conditions.[14,15] Since the normal liver dry weight is considered to be 20%–25% of the wet weight,[14,16] glycogen can comprise a sizable fraction of the total liver dry weight. Thus the influence of glycogen on liver ultrasonic properties warrants investigation because large molecular weight biomolecules can have high specific absorption,[17] and physiological levels of glycogen can approach a sizable fraction of total liver dry weight.

Inorganic phosphate has been shown theoretically and experimentally to increase the attenuation of protein solutions[17–19] by proton exchange relaxation mechanisms. Although physiological levels of inorganic phosphate are typically below 40 mM in various organs,[15] this concentration could be realized or exceeded in muscle tissue after anaerobic exhaustion, or after organ death when organic phosphates (i.e., ATP, ADP, AMP) are converted to the inorganic form over time. Thus, if inorganic phosphate measurably influenced liver attenuation, the result would be a time-varying component related to tissue energetics, similar to the case of glycogen. Our results show that glycogen can play a major contributing role to liver attenuation; however, the effect of additional phosphate appears to be insignificant at physiological concentrations.

I. MATERIALS AND TECHNIQUES

The glycogen for this study was obtained as bovine liver extract from the Aldrich Chemical Corp. and stored at 4 °C. The phosphate was a 50–50 mixture (by molarity) of monobasic and dibasic potassium phosphates, such that the *p*H remained close to 7.4.

Whole tissue samples were taken from previously frozen calf liver. The plain homogenate samples were obtained by mixing whole liver with an equal amount by weight of chilled, degassed distilled water in a blender for 2 min. The mixture was degassed in a 25- to 28-in. Hg vacuum for 10 min to eliminate bubbles, and then pipetted into a pillbox-shaped sample holder with clear, plastic-film acoustic windows. The homogenate was again degassed under vacuum for 20 min, and any remaining surface foam was skimmed off before covering and sealing the sample holder.

Glycogen and phosphate were dissolved slowly in distilled water to form the following solutions: 2-M, 1-M, and 0.2-M phosphate (30%, 15%, and 3% by weight) and 10%, 6%, and 4% glycogen solutions. These solutions were mixed with equal weight of whole liver to produce homogenized samples, processed as described above. The attenuation coefficient for each homogenate was multiplied by 2 to correct

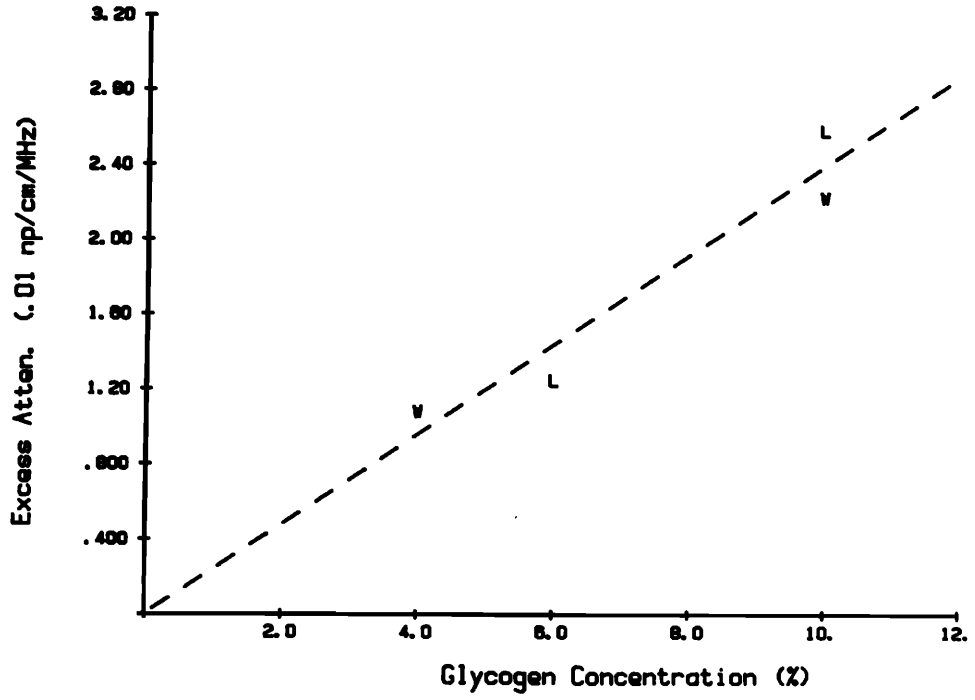

FIG. 1. Excess attenuation (divided by frequency, A/f) at 5 MHz versus glycogen concentration in distilled water (W) and liver homogenate (L). Dashed line is least-square-error linear curve fit. A/f (Np/cm/MHz) = 0.24 × concentration, where concentration is entered in units of g/ml.

for the dilution of liver and glycogen or phosphate.

The amplitude attenuation coefficient of the liver samples (with and without additives) was determined by a phase-insensitive radiation force technique as described in detail elsewhere.[8,9] Briefly, the sample, 1–2 cm thick, was inserted between a transducer and an absorbing target hung from a microbalance to measure insertion loss. Eight discrete frequencies were employed in the range of 1–12 MHz,[8,9] and all experiments were performed at room temperature, 20 °C–21 °C.

II. RESULTS

The excess attenuation divided by frequency for the added glycogen was linear with concentration (slope = 0.24-Np/cm/MHz/concentration at 5 MHz) in both distilled water and liver homogenate (Fig. 1). Figure 2 displays the frequency dependence of the attenuation coefficient (divided by frequency) of the liver homogenate (distilled water), a 10% glycogen solution, and 10% glycogen–liver homogenate. Coefficients for power-law fits are given in

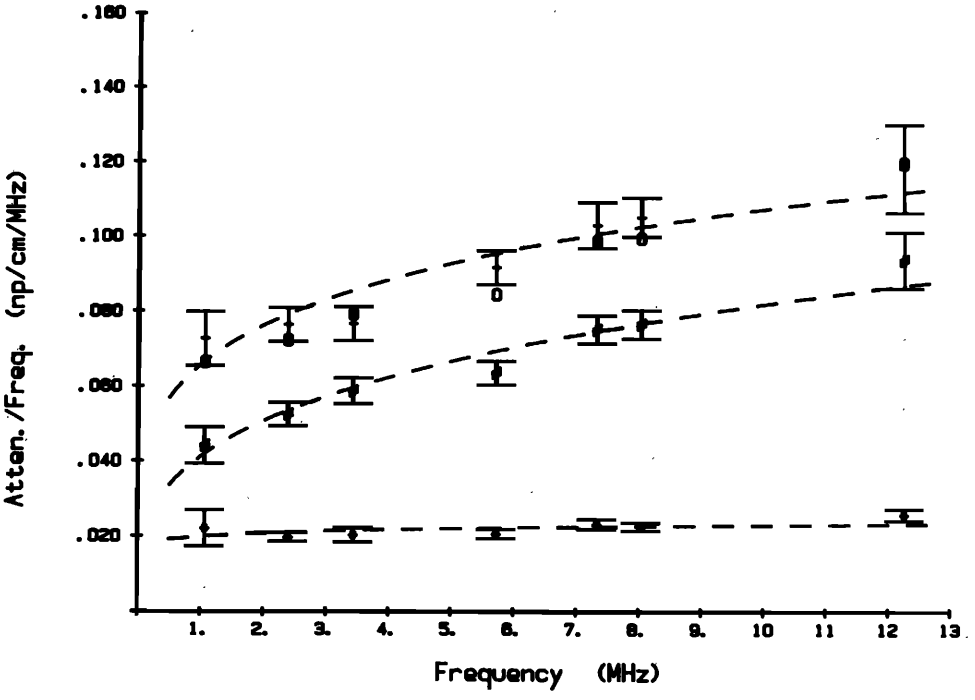

FIG. 2. Attenuation (divided by frequency, A/f) versus frequency for homogenized liver (#), homogenized liver with 10% glycogen (+), and 10% glycogen in distilled water (*). Circles indicate sum of liver and glycogen attenuation values. Error bars represent ± 1 s.d. Dashed lines represent curve fit to $A = \alpha_0 f^n$.

TABLE I. Coefficients of power-law fits ($A = \alpha_0 f^n$) for attenuation data.

	α_0 (Np/cm/MHz)	n
10% glycogen	0.020	1.067
Liver homogenate	0.041	1.300
Glycogen–liver homogenate	0.066	1.214
2-M phosphate	0.003	1.675
Liver homogenate	0.046	1.230
Phosphate–liver homogenate	0.056	1.300

Table I. The sum of the coefficients for the plain homogenate and the glycogen solution is also shown (circles) to demonstrate the additive property of the attenuation.

In contrast, Fig. 3 shows the frequency-dependent attenuation for a high concentration of phosphate, 2 M, added to a liver homogenate. Here, the liver–phosphate attenuation is statistically higher (to a 95% confidence level using Student's t-test) than the summed value for homogenate and phosphate (circles). Figure 4 compares the excess attenuation versus concentration for phosphate in both distilled water and liver homogenate. However, the attenuation coefficient for liver homogenate with more nearly physiological levels of phosphate (0.2 M) was indistinguishable from the distilled water homogenate within the experimental noise level of approximately 5%.

III. DISCUSSION

Glycogen, in distilled water or in liver homogenate, has a specific absorption coefficient (SAC) (Np/cm/concentration), at 10 MHz, equal to approximately 2.2 cm^2/g. Compared to large molecular weight proteins in distilled water, glycogen has a higher SAC than most studied by Kremkau and Cowgill,[17] and is comparable to the SAC of bovine serum albumin (2.05 cm^2/g), beta-lactoglobulin (1.81 cm^2/g), and ovalbumin (2.22 cm^2/g). The relatively high SAC and concentration of glycogen in liver can combine to produce a measurable influence on the organ's attenuation coefficient. Assuming mammalian liver in a resting, fasting state (glycogen $\leqslant 1\%$) has an attenuation coefficient of 0.30 Np/cm[2,3,6] at 5 MHz, then the addition of glycogen to 10% liver weight would raise the attenuation coefficient to 0.42 Np/cm at 5 MHz, a nearly 40% increase at abdominal imaging frequencies. This upper limit calculation assumes that no concomitant changes occur, such as water uptake, and cellular and organ swelling. Also, some differences in glycogen attenuation may result from "granular" storage in hepatocytes. The influences of these cofactors on *in vivo* attenuation are currently under investigation.

The mechanisms of absorption in glycogen solution deserve further attention. The SAC of glycogen is much higher than mono-, di-, tri-, and polysaccharides,[17] even though glycogen is formed via relatively simple concatenation of glucose (monosaccharide) molecules. The attenuation of glycogen solutions increases with frequency to the first power (approximately), indicating that a wide range of relaxation time constants must be responsible for the losses.[4,5,16]

The involvement of glycogen in liver attenuation poses both difficulties and opportunities for tissue characterization. The difficulties result from the time-varying nature of glycogen storage, which depends on the recent history of glucose uptake and energy output. Whereas fat and collagen buildup in livers might influence attenuation but change over a time scale of days to months, glycogen storage depletion or buildup can occur within hours, introducing possibly significant variability in the attenuation of normal livers. Opportunities arise because the glycogen handling capabilities of a liver relate to the underlying energetics of the organ and whole body; thus information related to glycogen levels

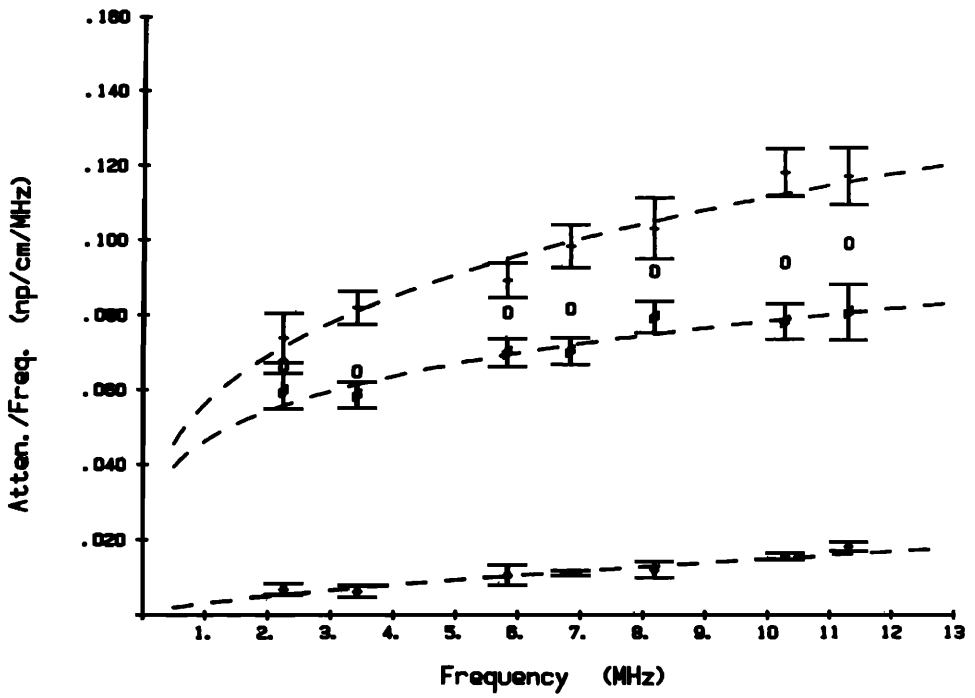

FIG. 3. Plot showing nonadditivity of attenuation for large phosphate concentrations. Attenuation (divided by frequency, A/f) versus frequency curves are for homogenized liver (#), homogenized liver with 2-M phosphate (+), and 2-M phosphate in distilled water (*). Circles indicate sum of liver and phosphate attenuation values, error bars are $\pm$ 1 s.d., and dashed lines represent curve fit to $A = \alpha_0 f^n$.

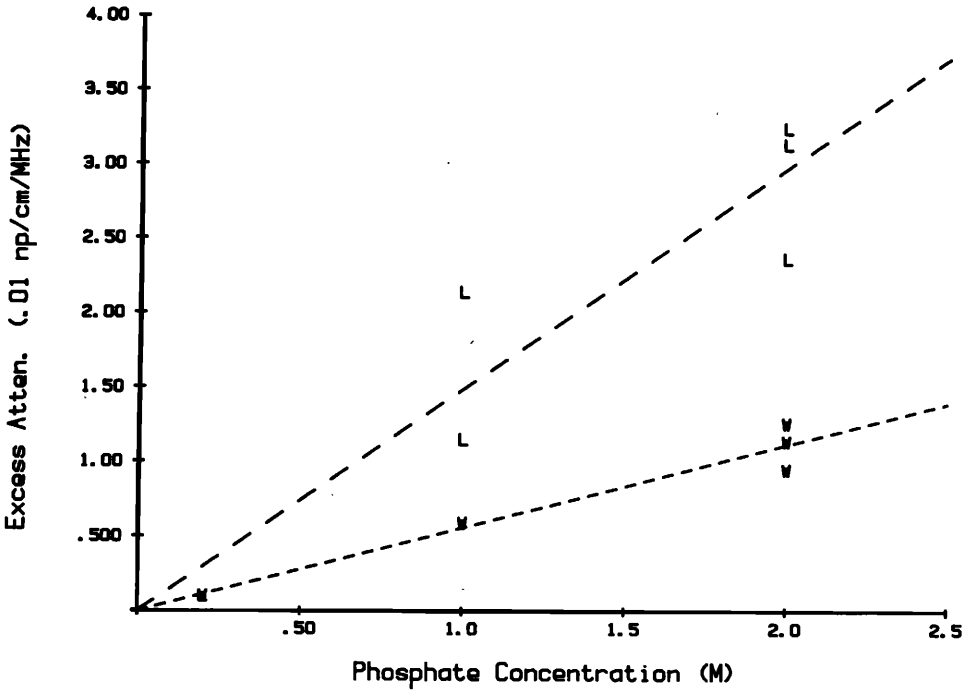

FIG. 4. Comparison of excess attenuation (divided by frequency, A/f) at 5 MHz versus concentration for phosphate in distilled water (W) and in liver (L). Linear curve fits are $A/f(\text{Np/cm/MHz}) = 0.0149 \times (\text{M})$ for phosphate in liver and $A/f = 0.0056 \times (\text{M})$ in water, where M is the molarity of the phosphate solution.

may be obtained noninvasively, under controlled conditions. Currently, special histology stains (PAS; periodic acid-Schiff) from liver samples are used to estimate glycogen levels. Glycogen accumulation is an important factor in diseases such as diabetes mellitus or hereditary enzyme deficiencies (glycogen storage diseases), and in toxicant-induced metabolic disease with disturbed carbohydrate metabolism. For example, dioxin in the guinea pig profoundly alters hepatocellular energy stores.[20–22] However, determination of glycogen levels as a function of time currently requires undesirable multiple liver biopsies. Thus glycogen assessment by ultrasonic parameter measurement would provide a noninvasive improvement.

The influence of phosphate on attenuation compares qualitatively with experimental observations of others.[17,19,23] Proton exchanges between inorganic phosphate and protein histidyl residues have been identified by Slutsky[18,23] as important absorption mechanisms. However, previous experiments have generally used purified proteins in much smaller (less than 5%) concentrations. In the case of our liver homogenate experiments, higher concentrations of proteins, amino acids, saccharides, and other compounds are already in solutions with physiological levels (presumably 4–40 mM) of inorganic phosphate, to which additional phosphate is added. The resulting excess attenuation was not measurable given the equipment used and the relatively high background attenuation, until unrealistically large phosphate concentrations were achieved. Since the precision of *in vitro* experimental conditions surpasses those likely to be encountered in clinical attenuation measurements,[24] the physiological fluctuations in liver phosphate levels (including postmortem changes) are not likely to measurably influence clinical attenuation estimates.

IV. CONCLUSIONS

Both glycogen and inorganic phosphate are involved in cellular and whole organ energy balance. Measurements of these compounds in liver homogenates and distilled water solutions show that fluctuations in the inorganic phosphate content of the liver are unlikely to produce measurable changes in the ultrasonic attenuation coefficient. In contrast, glycogen storage in liver may result in substantial time-varying changes in attenuation. The result is challenging for tissue characterization of diffuse liver diseases. If the effect of fat or other factors is to be studied, then the glycogen level must be a controlled variable. Alternatively, the possibility is raised of studying liver glycogen storage and glycogen disorders through measurements of attenuation as a function of time under controlled conditions.

ACKNOWLEDGMENT

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