

●Original Contribution

LIVER GLYCOGEN AND WATER STORAGE: EFFECT ON ULTRASOUND ATTENUATION

T. A. TUTHILL†, R. B. BAGGS‡ and K. J. PARKER†

†Rochester Center for Biomedical Ultrasound

‡Division of Laboratory Animal Medicine and Department of Pathology and Laboratory Medicine,
University of Rochester, Rochester, NY 14627

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Abstract—Glycogen has been shown *in vitro* to have a high specific absorption coefficient (ultrasound absorption in water per concentration) compared to other proteins. Depending on the amount of water which may accompany glycogen flux into and out of liver cells, the ultrasound attenuation coefficient of the liver may rise and fall with stored hepatic glycogen. This paper reports *ex vivo* studies on rats and *in vivo* studies on normal human volunteers before and after fasting. The results show a statistically significant difference in liver attenuation between well fed and fasted individuals. Generally, the attenuation difference is greater than 10%, and indicates that liver wet weight may not be strictly constant over glycogen storage cycles, as suggested in classic works. In contrast, no significant change in ultrasound backscatter is noted. The results point to the possible role of ultrasound attenuation measurements as a sensitive indicator of tissue physiology, and suggest that glycogen (feeding or fasting) must be controlled in tissue characterization experiments which compare liver attenuation coefficients of individuals and groups.

Key Words: Liver, Attenuation, Absorption, Tissue characterization, Ultrasound, Glycogen.

INTRODUCTION

Attenuation is viewed as a promising tissue characterization parameter in diagnostic ultrasound, yet its use is hindered by large measurement variations, presumably due to both biological variability and measurement uncertainties. In the search for possible causes of variations in attenuation, glycogen was chosen for analysis due to its fluctuating concentration in the liver. Upon digestion, glucose is taken up by the liver cells, polymerized, and stored as glycogen (Fawcett 1986). In response to the body's energy requirements, this glycogen is broken down and released into the bloodstream. Thus the total amount of glycogen in the liver can vary from 0%–10% (but more typically between 1%–4%) during the digestive cycle of normal adults.

In a preliminary study (Parker et al. 1988), glycogen was found to have a high specific absorption coefficient (SAC) compared to other high molecular weight biomolecules. Figure 1 shows a linear dependence of excess attenuation on glycogen concentra-

tion in distilled water and in liver homogenate (α/f [Np/cm-MHz] = 0.24 × concentration [g/cc]). According to this simple model, an increase in liver glycogen concentration from 0% to 10% of wet weight would raise the attenuation at 5 MHz by almost 50%, assuming no other changes occurred. However, *in vivo* glycogen storage is different in many respects from a mixed glycogen solution. In particular, the storage of glycogen in the liver can be accompanied by increases in water and potassium (Fenn 1939). To further examine effects of glycogen *in vivo*, ultrasound attenuation and backscatter were measured in normal and fasted livers. This follow-up study includes both *ex vivo* (through transmission in excised rat livers) and *in vivo* (attenuation of echo amplitudes in human livers) experiments.

METHODS

Fifteen white male Wistar rats (Charles River) were used to test the effect of fasting on liver attenuation. Seven of the rats were fasted for 24 hours with adequate water supply, and the remaining eight were fed *ad libitum* as controls. All were then euthanized with CO₂, and their livers immediately extracted and

Address all correspondence to: Kevin J. Parker, Dept. of Electrical Engineering, University of Rochester, Rochester, NY 14627.

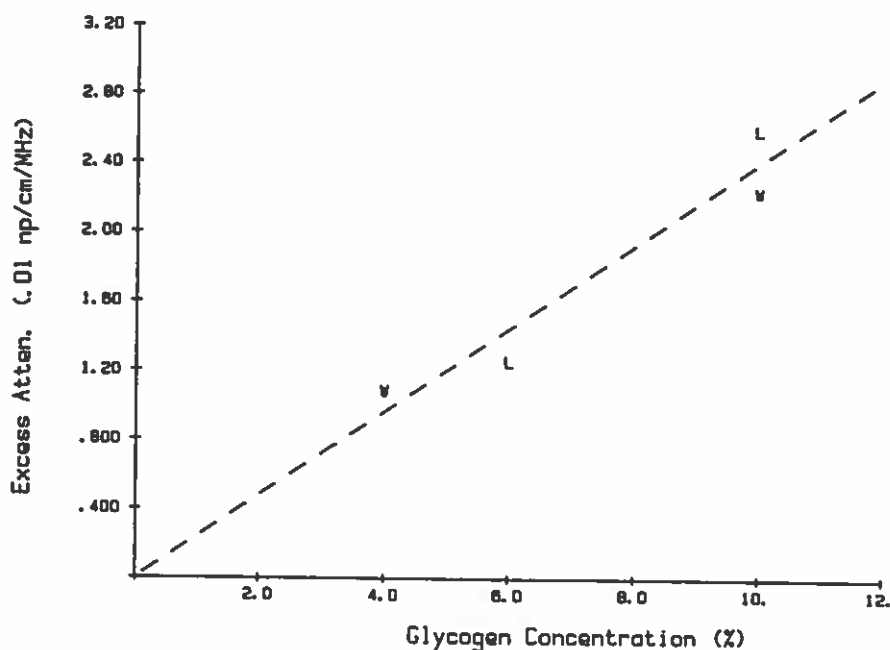


Fig. 1. Excess attenuation (divided by frequency, α/f) at 5 MHz vs. glycogen concentration in distilled water (*W*) and liver homogenate (*L*). The dashed line is the least-squares-error linear curve fit. α/f [Np/cm-MHz] = $0.24 \times$ concentration where concentration is in units of g/cc. Error bars on data points (not shown) are estimated to be $\pm 11\%$ standard deviation. [Reprinted with permission from Parker et al. 1988, p. 375.]

stored in saline at 4° C. Attenuation measurements were performed at 20° C within two hours of extraction using the radiation force technique described elsewhere (Lyons and Parker 1988). Briefly, within each group (fasted and controls) four whole excised livers were packed together in a disk-shaped specimen holder to form a uniformly (~2.5 cm) thick sample. Thin plastic wrap for the top and bottom coverings maintained relatively parallel surfaces and served as acoustic windows. The sample was then placed between a transducer and a rubber absorber contained in a large tank of degassed water at 20° C. Attenuation was determined from the difference between radiation force on the absorber with and without the sample in place. The frequency dependence of attenuation was determined over a range of 1–12 MHz by repeating the technique for three narrow band transducers at their fundamental and odd harmonics.

To also take into account changes in water content, the wet/dry weight of each liver was measured using a small sample (<1 cm³) cut from each. These specimens were weighed, heated at 90° C for 20 h, cooled in a dessicator, and reweighed.

A semi-quantitative assessment of glycogen was obtained from the periodic acid-Schiff (PAS) stain applied to histology specimens and ranked on a scale of 0–4⁺ according to density by an experienced ex-

aminer (R.B.B.). Using literature data for comparison, we presume that a rating of 0 corresponds to less than 1% glycogen, while a score of 3⁺ or higher corresponds to more than 4% hepatic glycogen.

The clinical study involved scanning eight male, "normal" volunteers, aged 25–33 years, at two presumably different liver glycogen levels. Volunteers were non-obese, had no medical history of liver disease, were not on any prescription drugs, and did not consume alcohol in the week prior to the study. Each volunteer was encouraged to eat high carbohydrate, low fat meals for two days before the initial scans. The volunteers were referred to guidelines from "carbohydrate loading" discussions in popular sports literature. Scanning was repeated 18 hours later, during which time the volunteers fasted or ate a very light meal, such as a salad. Normal water consumption was encouraged. Volunteers were scanned using an approved protocol after informed consent had been obtained. Data from the clinical scanner (Technicare annular array, 3.5 MHz center frequency) was digitized (20 MHz sampling) and stored on tape for later processing. Three liver scans (2 longitudinal, 1 transverse) were made for each volunteer, on each day, along with two phantom scans which served as a check on the long and short term stability of the instrument. Images were derived from the analytic envelopes of the received signals, and effects of time-

varying gain and beam diffraction were removed (Parker *et al.* 1984; Tuthill *et al.* 1988).

Specific regions of interest (ROIs) were chosen from each scan for analysis using the following guidelines.

1. Large visible specular reflections were avoided.
2. Minimum allowable dimensions were length ≥ 2 cm (≥ 500 pts) and width ≥ 0.75 cm (≥ 15 lines). Relatively long data lines were sought since uncertainties of attenuation estimates are reduced more with increased range than with increased width (Parker 1986).
3. The signal-to-noise ratio (mean speckle amplitude to standard deviation) within the ROI must be greater than 1.5 to ensure a relatively homogenous speckle pattern and to eliminate the possibility of additional specular reflectors (Tuthill *et al.* 1988).
4. Liver ROIs must be deep enough to avoid the presence of "ring down" from the skin/fat layers.
5. Liver ROIs could not be too deep such that actual attenuated RF signals approached noise levels.

The received ultrasound signal envelope, $S(x)$, is assumed to attenuate exponentially with the form,

$$S(x) = s_0 e^{-2\alpha x} \quad (1)$$

where x represents range and s_0 is a constant representing the backscattered signal strength from the ROI. The tissue attenuation coefficient, α , was estimated for each ROI by averaging envelopes of adjacent scan lines and then curve-fitting to an exponen-

tial (Fig. 2). A least-squares linear fit was used after taking the natural log of eqn (1) and applying the appropriate weighting factors (Bevington 1969). The decay of the envelope was taken to indicate the attenuation of the center frequency of the pulse, 3.5 MHz in our case. The results in Np/cm were divided by 3.5 MHz to give a result in Np/cm-MHz.

Four to six acceptable ROIs were found in each scan, and the average attenuation coefficient was computed from among all three scans for a total of at least twelve ROIs averaged per individual. The mean backscatter amplitude (after correcting for attenuation in overlying tissue) was also determined for each ROI.

RESULTS

A preliminary study of 16 rats showed that those fed *ad libitum* ($N = 8$) had an average PAS score of 2.8⁺, whereas those fasted for 24 h ($N = 8$) had an average PAS score of 0.2⁺. Black and white photographic reproductions of the colored stained sections are shown in Fig. 3. These reproductions were made at constant exposure to maintain the relative densities of the slides. Although the stain results were semi-quantitative, they were in rough agreement with Fenn's (1939) measurement of 4% liver glycogen in fed rats and 0.2% glycogen in 48 h fasted rats.

The frequency-dependent attenuation results for fasted and normal rat livers are shown in Fig. 4. Coefficients for power law fits are given in Table 1. The fasted livers had a 10%–17% lower attenuation over

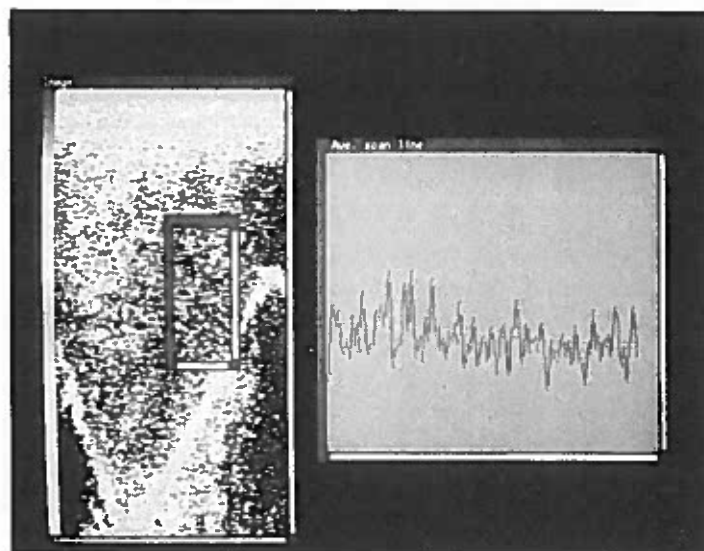
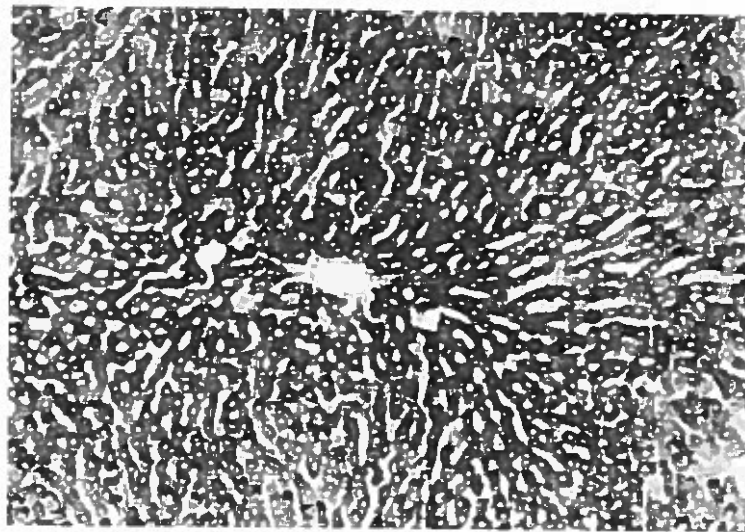


Fig. 2. Left: Liver scan (transducer insonifying from top) with box demarcating region of interest (ROI) used to determine the attenuation coefficient. Right: Scan lines are averaged across the width of the ROI and curve fit to an exponential (white line).



(a)



(b)

Fig. 3. Black and white photographic reproductions of colored PAS stained sections of rat livers both normal (a) and fasted (b).

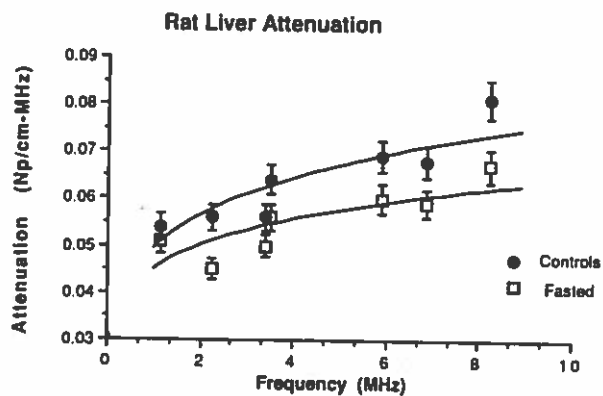


Fig. 4. Attenuation (divided by frequency, α/f) vs. frequency for normal and fasted rat livers. Error bars represent ± 1 *sd*. Solid line represents curve fit to $\alpha = \alpha_0 f^n$.

all frequencies. Their water concentration, however, was $73.0\% \pm 1.2\%$, significantly above the $71.0\% \pm 0.7\%$ found in normals. When the attenuation coefficients are normalized by water content in the calculation of SAC, the results in Table 1 show a slightly higher SAC for the glycogen filled livers. However, the 7% difference is within the 7%–10% estimated error in the measurement technique (Lyons and Parker 1988).

In seven out of the eight volunteers in the clinical study, attenuation dropped 10%–20% after fasting, as shown in Fig. 5. Values for volunteer No. 6 remained essentially unchanged. The changes in total average attenuation can be contrasted with the average attenuation of the reference phantom scanned on each

Table 1. Coefficients of power law fits ($\alpha = \alpha_0 f^n$) for rat liver attenuation and specific absorption coefficient (SAC) at 5 MHz.

	Attenuation		SAC _{5MHz/5 MHz} (cm ² /g-MHz)
	α_0 (Np/cm-MHz ⁿ)	<i>n</i>	
Normals	0.048	1.21	0.232
Fasted	0.045	1.16	0.216

day. The increase in the reference attenuation may be explained by the temperature dependence of the phantom. The "glycogen day" scans were taken on Friday afternoons when the clinical area was warm from equipment heat dissipation whereas the "fasted day" scans were taken on Saturday mornings when the room temperature was approximately 3° C lower. The repeatable difference of 0.005 Np/cm-MHz is consistent with independent measurements of phan-

tom attenuation over 20 °C–26 °C. A paired *t* test on all volunteers showed a significant drop in attenuation at the 5% level ($p < 0.05$). In comparison, there was no significant difference in the mean backscatter values of livers measured before and after fasting (Fig. 6).

DISCUSSION

Fasting influences liver attenuation, though the actual role of glycogen can only be indirectly assumed in *in vivo* studies. If glycogen was removed as inert matter without any other changes in liver water content, then a drop in glycogen concentration from 5% of liver wet weight to 0% wet weight would decrease the attenuation by 0.012 Np/cm-MHz or approximately 25% of the normal level (0.05 Np/cm-MHz). While glycogen levels in the liver are depleted during fasting, other liver components, such as water concentration, are also affected. Fenn (1939) found that

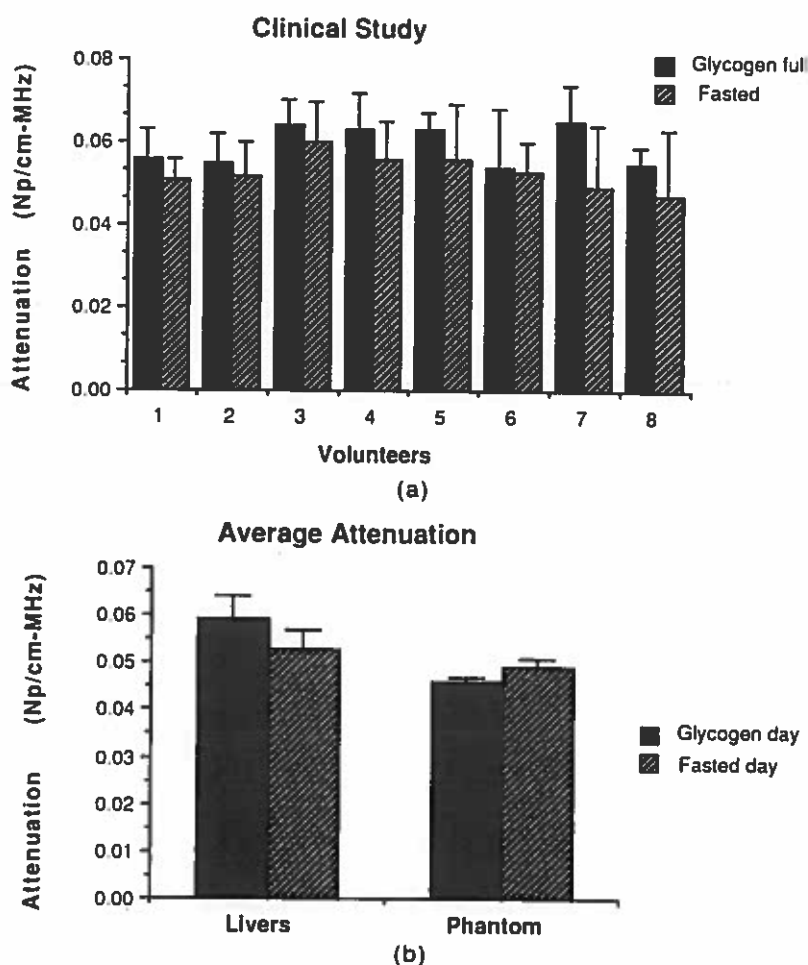


Fig. 5. (a) Attenuation values for each volunteer determined from clinical ultrasound imaging before and after fasting. (b) Average attenuation values of all volunteers before and after fasting as compared with the reference phantom scanned on each day. Error bars represent 1 *sd*.

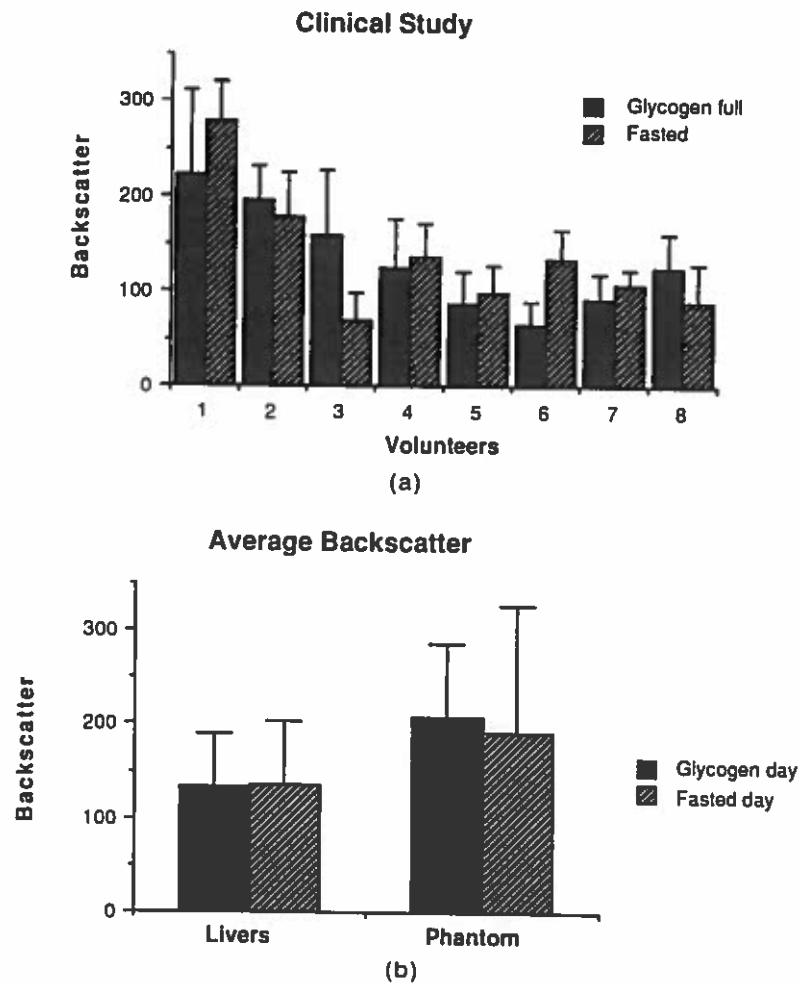


Fig. 6. (a) Mean backscatter values (arbitrary units) for each volunteer determined from clinical ultrasound imaging before and after fasting. (b) Average backscatter values of all volunteers before and after fasting as compared with the reference phantom scanned on each day. The phantom had a high backscatter value, so for this figure, the phantom averages were divided by 4.

in fasted rats the total liver weight decreased and glycogen was removed with sufficient water to maintain relatively constant liver density and wet/dry ratio. A decrease in water could tend to counteract the loss of glycogen producing a smaller change in overall attenuation than would be the case if glycogen alone was removed. While Fenn concluded that sufficient water is transported with glycogen to maintain a constant dry/wet weight ratio (contrary to our rat study), it should be noted that his experiment involved fasting rats and then refeeding them with a liquid sugar diet. The possibility of glycogen being taken up without its full complement of water during "solid" carbohydrate loading in humans remains open.

To quantitatively demonstrate the effects of changes in glycogen and water content, the liver attenuation was modeled as a linear sum of attenuation contributions from each tissue component,

$$\alpha = \alpha_G C_G + \alpha_O C_O \quad (2)$$

where α is the specific absorption coefficient (SAC), C is the concentration, and the subscripts correspond to glycogen (G), and proteins with any other components (O). Using measured values of α_G , α_O , and water concentrations, and also estimating glycogen concentrations from our PAS stains and Fenn's (1939) results, we can calculate a predicted attenuation for rat livers in different states as shown in Table 2. In these calculations we have assumed that weight percent (g/g) is approximately equal to concentration (g/cm^3), assuming in all cases that liver density remains close to $1 \text{ g}/\text{cm}^3$. The results compare well with measured values. It is important to note that since the SACs of glycogen and liver proteins are similar, the glycogen-to-protein balance of solid weight is far less influential on attenuation than the total wet to

Table 2. Components of attenuation in rat livers.

$$\alpha = \alpha_G C_G + \alpha_O C_O; \quad \alpha_G = 0.223^{(1)}; \quad \alpha_O = 0.216^{(2)}$$

	Water content ⁽³⁾	Glycogen conc. ⁽⁴⁾	Other conc. ⁽⁵⁾	Atten. _{5 MHz} /5 MHz (Np/cm-MHz)	
				Calculated	Measured ⁽⁶⁾
Normal	71.0%	4.0%	25.0%	0.064	0.067
Fasted	73.0%	0.2%	26.8%	0.058	0.058

⁽¹⁾ Measured from Parker *et al.* (1988).

⁽²⁾ Measured from fasted livers and consistent with protein SAC (Kremkau and Cowgill 1984).

⁽³⁾ Measured using wet/dry ratio.

⁽⁴⁾ Assumed from our PAS staining and Fenn's (1939) data.

⁽⁵⁾ Sum to 100% in each case.

⁽⁶⁾ Using curve fit over all frequencies and evaluating at 5 MHz.

dry ratio. That is, if glycogen-protein amounts are 4% and 25% or 5% and 24% respectively, there is little difference in final attenuation. A major difference does occur if their sum is changed from 29% to 27% as in the case of fasted livers. Thus, water content is the more influential component of attenuation changes, but glycogen flux appears to be one mechanism by which the SAC of liver and the water content change simultaneously. It is also interesting to note that the changes in composition from normal to fasted could be explained by removing 4 g glycogen and 4 g of water from 100 g of liver. After this subtraction, the remaining 92 g have a percent concentration of 73% water, 0% glycogen and 27% other, comparable with our estimated data (see Table 2).

In the human study, it must be emphasized that our volunteers were encouraged to follow a feeding/fasting regimen with intake of adequate, normal quantities of water. However, there were no strict controls or measures on the foods consumed or on exercising and activities during the fasting and feeding periods. Also, there were no practical means of directly measuring changes in their liver water and glycogen concentrations. Given these drawbacks, it is perhaps surprising to find the consistent and significant changes in attenuation. More dramatic results may be obtained with stricter "dietary" regimens, or in cases of abnormal hepatic glycogen and water storage due to toxic injury or disease.

CONCLUSION

Glycogen levels within the liver can have a significant effect on ultrasound attenuation. During fasting, glycogen is removed along with some water,

though the net solid concentration is decreased slightly in rats. The small difference allows for a measurable drop in attenuation. This variation within normal individuals may explain part of the wide range of attenuation coefficients found in the literature, and guidelines or controls on eating and activity before ultrasound scanning should be considered in future clinical studies. This demonstrates that ultrasound is a sensitive indicator of changes in a basic metabolic process, and opens new possibilities for diagnostic procedures.

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