Comparison of quantitative ultrasound parameters for fat content liver detection and monitoring

Pauline Muleki-Seya, Aiguo Han, *Member, IEEE,* Michael P. Andre, John W. Erdman, Jr., and William D. O'Brien, Jr., *Life Fellow, IEEE* E-mail: wdo@uiuc.edu

Abstract—Quantitative ultrasound parameter estimates from the Lizzi-Feleppa (LF slope, intercept and midband) and quantitative ultrasound (mean BSC, mean AC, ESD and EAC) approaches were compared for *in vivo* and *ex vivo* mouse liver fat content monitoring. The quantitative ultrasound parameters having the higher correlations with fat content were EAC (R^2=0.68), mean BSC (R^2=0.60), mean AC (R^2=0.60) and LF midband (R^2=0.70) for *ex vivo* conditions and mean BSC (R^2=0.45), LF midband (R^2=0.44) and mean AC (R^2=0.37) for *in vivo* conditions. By defining a threshold fat content of 11.6% between normal vs. fatty mouse livers, the parameters yielding the better sensitivities and specificities to differentiate normal and fatty liver *ex vivo* were the mean AC (sensitivity=1, specificity=1), then mean BSC and LF midband (sensitivity=0.87, specificity=1). *In vivo*, the parameters yielding the better sensitivities and specificities to differentiate normal vs. fatty liver were mean BSC and LF midband (sensitivity=0.93, specificity=0.87) and then the mean AC (sensitivity=0.67, specificity=0.93).

1 Introduction

There are two generally used ultrasonic approaches to extract sets of quantitative parameters: the Lizzi-Feleppa (LF) and quantitative ultrasound (QUS) approaches. The LF approach estimates LF parameters from the linear fit of the backscatter coefficient (BSC) versus frequency, and yields slope, intercept and midband. The QUS approach relies on the attenuation coefficient (AC) and BSC versus frequency, and yields the mean AC and mean BSC over a defined bandwidth. By using the spherical Gaussian model, QUS-derived parameters are estimated: the effective scatterer diameter (ESD) and effective acoustic concentration (EAC).

- *P. Muleki-Seya, A. Han and W. D. O'Brien, Jr. are with the Bioacoustics Research Laboratory, Department of Electrical and Computer Engineering, University of Illinois, 405 N. Mathews, Urbana, IL 61801.*
- *M. P. Andre is with the Department of Radiology, University of California at San Diego, 9500 Gilman Drive, San Diego, CA 92093.*
- *J. W. Erdman, Jr. is with the Department of Food Science and Human Nutrition, University of Illinois at Urbana-Champaign, 905 S. Goodwin, Urbana, IL. 61801.*

LF, QUS or QUS-derived parameters have been used for multiple applications such as the differentiation among cancer and healthy tissue [1], [2] or between cell types [3], [4], the detection or monitoring of fat in liver [5], [6] and the monitoring of anti-cancer therapy [7], [8]. It is thus of great importance to determine if one or another parameter may be more appropriate to use for each application. In this study, the correlation between these parameters and liver fat content is studied, as well as their sensitivity and specificity to differentiate among normal and fatty mouse livers under *in vivo* and *ex vivo* conditions.

The goal of this paper is to examine which parameter(s) is(are) better for estimating liver fat content, and to distinguish between normal and fatty livers.

2 Material and Method

2.1 Animal protocol

The ultrasound data for this study were acquired from *in vivo* and freshly excised livers of C57BL/6J mice. For each mouse, the ultrasound data were acquired *in vivo* transabdominally under anesthesia prior to euthanasia. The ultrasound data from freshly excised livers were then acquired. The 30 mice were fed either a control (n=15) or a high-fat (n=15) diet, as detailed in [5]. After ultrasonic RF data acquisition, the liver fat percentage was estimated by the Folch biochemical lipid assay and yielded a range from 4% to 24%.

2.2 Ultrasound measurements

Liver RF data were acquired *in vivo* transabdominally using the VisualSonics Vevo 2100 with the MS-400 (12-33 MHz) array transducer. The freshly excised liver was placed in a saline bath at ambient temperature for *ex vivo* scanning with a 40 MHz f/3 single-element

transducer. For each ultrasonic image, a Field of Interest (FOI) containing the most homogeneous part of the liver was defined.

2.2.1 Attenuation and BSC in vivo

The attenuation for each liver was obtained using the spectral difference reference phantom method [9], with implementation details described in [5]. The attenuation was estimated for each sub-ROI (1.5 x 1.5 mm) within the FOI and averaged to obtain the mean attenuation (dB/cm) versus frequency curve over the -10 dB bandwidth 12 - 33 MHz. The BSC was estimated using the reference phantom method [9], as described in [5]. The BSC was estimated for each sub-ROI (1.5 x 1.5 mm) within the FOI and averaged to yield the mean BSC versus frequency curve over the -10 dB bandwidth 12 - 33 MHz.

2.2.2 Attenuation and BSC ex vivo

The attenuation for each liver was obtained using a broadband insertion-loss technique [10], [11] with a 40-MHz f/3 focused transducer, with methodology described in [12]. The attenuation was estimated from 36 independent lateral locations across the sample and averaged to obtain the mean attenuation (dB/cm) versus frequency curve over the -10 dB bandwidth 25 - 55 MHz. The BSC was estimated using the planar reference technique [13], as described in [5]. The BSC was estimated for each sub-ROI within the FOI (equivalent to 15 x 15 wavelengths at 40 MHz) and averaged to yield the mean BSC versus frequency curve over the -10 dB bandwidth 25 - 55 MHz.

2.3 Parameters definition

BSC and AC were averaged *in vivo* over the 18-28 MHz bandwidth and *ex vivo* over the 33-47 MHz bandwidth to yield the respective mean BSC and mean AC. The LF parameters were estimated from the best linear fit on $10log(BSC)$ versus linear frequency: slope, intercept at 0 MHz and midband fit (amplitude in dB at 22.5-MHz *in vivo*/40-MHz *ex vivo* central frequency). The QUS-derived parameters correspond to scatterer parameters and were extracted by fitting the BSC with a theoretical BSC model using the Gaussian form factor. The method to obtain the scatterer estimates (ESD and EAC) is presented in [14]. ESD was estimated from values that ranged between 1 and 100 μm with a step size of 1 μm. When ESD was found equal to 1 μm, the fit was considered incorrect and the QUS-derived values were not taken into account.

3 Results

3.1 Correlations between LF, QUS and QUS-derived parameters and fat content

The correlations between the LF, QUS and QUS-derived parameters and the liver fat content are presented in Figure 1 for *ex vivo* conditions and in Figure 2 for *in vivo* conditions with the best linear fits. The corresponding correlation coefficients are summarized in Table 1. *Ex vivo*, the parameters presenting the better correlations with fat content are EAC (R^2=0.68), mean BSC and mean AC (R^2=0.60) and LF midband (R^2=0.55). *In vivo*, these correlation coefficients are lower; the parameters presenting the better correlations with fat content are mean BSC (R^2=0.45), LF midband (R^2=0.44) and mean AC (R^2=0.37).

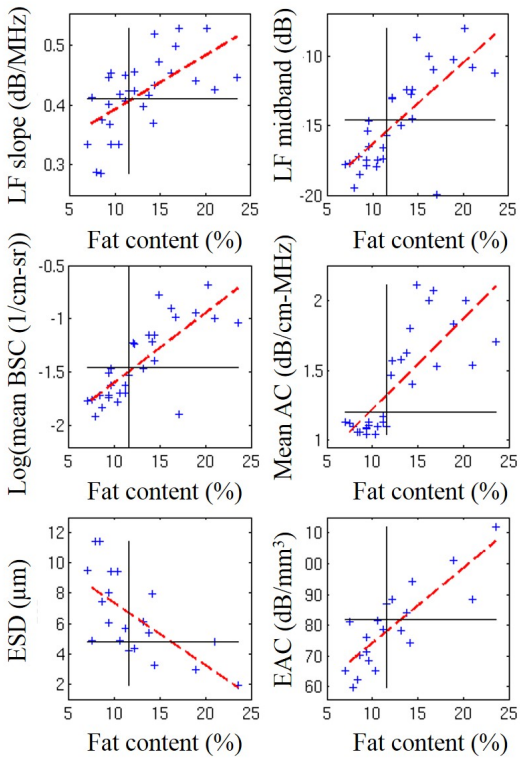

Fig. 1. LF (slope and midband), QUS (mean BSC and mean AC) and QUS-derived (ESD and EAC) parameters as a function of fat percentage for *ex vivo* conditions. The red dashed lines correspond to the best linear fits. The black lines correspond to a fat percentage threshold of 11.6 % and to LF, QUS and QUS-derived thresholds defined to separate normal from fatty livers.

3.2 Normal/fatty liver differentiation

The fat content for fatty liver disease in humans is typically defined as greater than 5% [6]. However, for mice there is no defined fat content threshold for fatty liver disease because this disease is not defined in mice. In this study therefore, normal mouse liver

R^2	LF slope	LF intercept	LF midband	log(BSC)	AC	ESD	EAC
fat percentage (*ex vivo*)	0.38	0.10	0.55	0.60	0.60	0.45	0.68
fat percentage (*in vivo*)	0.16	0.03	0.44	0.45	0.37	0.05	0.22

TABLE 1
Correlation coefficients between the LF, QUS and QUS-derived parameters estimated from *ex vivo* and *in vivo* BSC with the fat percentage for linear fits.

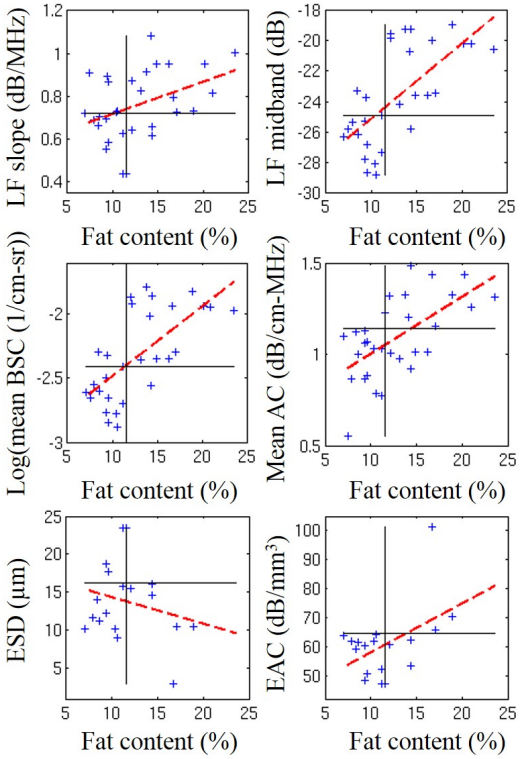

Fig. 2. LF (slope and midband), QUS (mean BSC and mean AC) and QUS-derived (ESD and EAC) parameters as a function of fat percentage for *in vivo* conditions. The red dashed lines correspond to the best linear fits. The black lines correspond to a fat percentage threshold of 11.6 % and to LF, QUS and QUS-derived thresholds defined to separate normal from fatty livers.

is defined to correspond to a fat content $<$ 11.6% and fatty liver to fat content $\geq$ 11.6%. This 11.6% threshold corresponds to the maximum fat content obtained for mice that were fed the control diet. Based on this fat content threshold, LF, QUS and QUS-derived parameter thresholds were defined to separate fatty from normal liver. These thresholds, obtained using the highest overall combination of sensitivity and specificity (Youden index), are shown on Figures 1 and 2 for *ex vivo* and *in vivo* conditions, respectively, and are summarized in Table 2. These thresholds are used to estimate sensitivity and specificity of LF, QUS and QUS-derived parameters. The results are presented in Table 2 for *ex vivo* and *in vivo* conditions. The parameters yielding the better sensitivities and specificities to differentiate normal vs. fatty liver *ex vivo* are mean AC (sensitivity=1, specificity=1), then mean BSC (sensitivity=0.87, specificity=1) and LF midband (sensitivity=0.87, specificity=1). *In vivo*, the parameters yielding the better sensitivities and specificities to differentiate normal vs. fatty liver are mean BSC and LF midband (sensitivity=0.93, specificity=0.87).

4 Discussion

The goal of this study was to determine which quantitative ultrasound parameters among LF, QUS and QUS-derived parameters presented better correlations with liver fat content, as well as their sensitivity and specificity to differentiate among normal and fatty liver for *in vivo* and *ex vivo* experimental conditions.

The parameters presenting the better correlations with liver fat content were mean BSC, mean AC, LF midband and EAC (only for *ex vivo* conditions). These parameters are related to amplitudes of BSC versus frequency with mean BSC and LF midband corresponding to the amplitude of $log_{10}(BSC)$ and $10log_{10}(BSC)$ around and at the central frequency, respectively; EAC was estimated from a linear fit of $10log(BSC)$ and mean AC had an impact on the BSC amplitude. Note that the parameters related to BSC slope, LF slope and ESD, present a lower correlation with fat content. A parameter related to BSC amplitude is thus better to monitor liver fat content. EAC presents a good correlation with liver fat content *ex vivo*. However, in this study a significant number of ESD/EAC values were removed because ESD values corresponded to the lower value of the range used for the estimation and were not considered correct (only 20 ESD values for *ex vivo* experimental conditions and 18 ESD values for *in vivo* experimental conditions out of 30 were considered correct).

By defining that the mouse liver fat content threshold between normal and fatty livers was 11.6%, thresholds for the different parameters were defined in order to obtain the maximum of the average of sensitivity and specificity values for each parameter. The parameters yielding the better sensitivities and specificities were mean AC (*ex vivo* conditions), mean BSC and LF midband. Except for EAC (*ex vivo* conditions), these parameters were the ones providing the better

	ex vivo			*in vivo*		
	sensitivity	specificity	threshold	sensitivity	specificity	threshold
LF slope	0.87	0.67	0.41 dB/MHz	0.80	0.67	0.72 dB/MHz
LF intercept	1	0.07	-41.0 dB	1	0	-48.3 dB
LF midband	0.87	1	-14.6 dB	0.93	0.87	-24.9 dB
log(mean BSC)	0.87	1	-1.45 log(1/cm-sr)	0.93	0.87	-2.4 log(1/cm-sr)
mean AC	1	1	1.2 dB/cm-MHz	0.67	0.93	1.1 dB/cm-MHz
ESD	0.92	0.63	4.8 μm	0.33	1	16.2 μm
EAC	0.75	0.92	81.9 dB/mm^3	0.50	1	64.6 dB/mm^3

TABLE 2
Sensitivity and sensibility to detect normal and fatty livers for *ex vivo* and *in vivo* LF, QUS and QUS-derived parameters. The fat percentage threshold is defined as 11.6 % and the LF, QUS and QUS-derived parameters threshold are summarized in the Table.

correlations with the liver fat content.

There were two major differences between *ex vivo* and *in vivo* outcomes: the attenuation methods are different and interposed tissues between skin and liver were present *in vivo*, but not *ex vivo*. Both differences may lead to errors in the evaluation of attenuation and BSC. An error in attenuation estimates would also yield impact on the BSC estimates through attenuation compensation. The insertion-loss technique used *ex vivo* provided more precise attenuation estimates than the spectral difference reference phantom method used *in vivo*. It was, thus, not surprising to obtain better correlation coefficients between LF, QUS and QUS-derived parameters with the liver fat content from *ex vivo* condition. The thresholds on the LF, QUS and QUS-derived parameters used to differentiate among normal and fatty livers were different for *ex vivo* and *in vivo* conditions. This difference may be related to errors in attenuation estimates *in vivo* or to the difference in the acquisition frequency (22.5 MHz *in vivo* and 40 MHz *ex vivo*).

5 Conclusion

For liver fat content monitoring or to differentiate among normal or fatty liver, mean BSC and LF midband provided the better results among the LF, QUS and QUS-derived parameters. Mean AC provided the better results *ex vivo* but seemed to be more affected by errors from the attenuation method or by the presence of interposed tissue between skin and liver *in vivo*.

Acknowledgments

The authors would like to thank J. R. Kelly, MS, and R. J. Miller, DVM, from the Bioacoustics Research Laboratory for the mouse liver preparation. This study was supported by NIH grant R37EB002641.

References

[1] E. J. Feleppa, F. L. Lizzi, D. J. Coleman, and M. M. Yaremko, "Diagnostic spectrum analysis in ophthalmology: a physical perspective," *Ultrasound in Medicine & Biology*, vol. 12, no. 8, pp. 623–631, 1986.

[2] J. Mamou, A. Coron, M. L. Oelze, E. Saegusa-Beecroft, M. Hata, P. Lee, J. Machi, E. Yanagihara, P. Laugier, and E. J. Feleppa, "Three-dimensional high-frequency backscatter and envelope quantification of cancerous human lymph nodes," *Ultrasound in Medicine & Biology*, vol. 37, no. 3, pp. 345–357, 2011.

[3] J. Mamou, M. L. Oelze, W. D. O'Brien Jr., and J. F. Zachary, "Ultrasound characterization of three animal mammary tumors from three-dimensional acoustic tissue models," in *Proceedings of the 2005 IEEE Ultrasonics Symposium*, 2005, pp. 866–869.

[4] M. L. Oelze and W. D. O'Brien Jr., "Application of three scattering models to characterization of solid tumors in mice," *Ultrasonic Imaging*, vol. 28, no. 2, pp. 83–96, 2006.

[5] A. Han, J. W. Erdman Jr., D. G. Simpson, M. P. Andre, and W. D. O'Brien Jr., "Early detection of fatty liver disease in mice via quantitative ultrasound," in *2014 IEEE International Ultrasonics Symposium*. IEEE, 2014, pp. 2363–2366.

[6] S. C. Lin, E. Heba, T. Wolfson, B. Ang, A. Gamst, A. Han, J. W. Erdman Jr., W. D. O'Brien Jr., M. P. Andre, C. B. Sirlin, and R. Loomba, "Noninvasive diagnosis of nonalcoholic fatty liver disease and quantification of liver fat using a new quantitative ultrasound technique," *Clinical Gastroenterology and Hepatology*, vol. 13, no. 7, pp. 1337 – 1345.e6, 2015.

[7] M. Kolios, G. Czarnota, M. Lee, J. W. Hunt, and M. D. Sherar, "Ultrasonic spectral parameter characterization of apoptosis," *Ultrasound in Medicine & Biology*, vol. 28, no. 5, pp. 589 – 597, 2002.

[8] A. Sadeghi-Naini, N. Papanicolau, O. Falou, J. Zubovits, R. Dent, S. Verma, M. Trudeau, J.-F. Boileau, J. Spayne, S. Iradji *et al.*, "Quantitative ultrasound evaluation of tumor cell death response in locally advanced breast cancer patients receiving chemotherapy," *Clinical Cancer Research*, vol. 19, no. 8, pp. 2163–2174, 2013.

[9] L. X. Yao, J. A. Zagzebski, and E. L. Madsen, "Backscatter coefficient measurements using a reference phantom to extract depth-dependent instrumentation factors," *Ultrasonic Imaging*, vol. 12, no. 1, pp. 58–70, 1990.

[10] K. A. Wear, T. A. Stiles, G. R. Frank, E. L. Madsen, F. Cheng, E. J. Feleppa, C. S. Hall, B. S. Kim, P. Lee, W. D. O'Brien Jr. *et al.*, "Interlaboratory comparison of ultrasonic backscatter coefficient measurements from 2 to 9 MHz," *Journal of Ultrasound in Medicine*, vol. 24, no. 9, pp. 1235–1250, 2005.

[11] R. Kuc, "Clinical application of an ultrasound attenuation coefficient estimation technique for liver pathology characterization," *IEEE Transactions on Biomedical Engineering*, no. 6, pp. 312–319, 1980.

[12] A. Han, M. P. Andre, J. W. Erdman Jr., R. Loomba, C. B. Sirlin, and W. D. O'Brien Jr., "Repeatability and reproducibility of a clinically based qus phantom study and methodologies," *IEEE Trans. Ultrason. Ferroelectr. Freq. Control*, vol. 64, no. 1, pp. 218–231, 2016.

[13] X. Chen, D. Phillips, K. Q. Schwarz, J. G. Mottley, and K. J. Parker, "The measurement of backscatter coefficient from a broadband pulse-echo system: A new formulation," *IEEE Transactions on Ultrasonics, Ferroelectrics, and Frequency Control*, vol. 44, no. 2, pp. 515–525, 1997.

[14] M. L. Oelze, J. F. Zachary, and W. D. O'Brien Jr., "Parametric imaging of rat mammary tumors in vivo for the purposes of tissue characterization," *Journal of Ultrasound in Medicine*, vol. 21, no. 11, pp. 1201–1210, 2002.