In vivo ultrasonographic exposimetry: Human tissue–specific attenuation coefficients in the gynecologic examination

Tariq A. Siddiqi, MD,[a] Menachem Miodovnik, MD,[a] Richard A. Meyer, MD,[b] and William D. O'Brien, Jr, PhD[c]

Cincinnati, Ohio, and Urbana, Illinois

OBJECTIVE: The purpose of the current study was to determine in vivo, tissue-specific ultrasonic attenuation coefficients for each of the tissue layers comprising the anterior abdominal wall, uterus, and vagina with use of a quantitative multilayer tissue model. We wanted to validate the "homogeneous" tissue model-based Food and Drug Administration derating factor of 0.3 dB/cm-MHz applied to obstetric-use ultrasonography systems.

STUDY DESIGN: With use of a 3.0-MHz mechanical sector scanner and our previously tested exposimetry equipment, we obtained a set of at least 5 separate acoustic pressure waveforms from each test subject by placing a calibrated 7-element linear-array hydrophone in the anterior vaginal fornix while she was undergoing transabdominal ultrasonography. Corresponding sets of reference in vitro acoustic pressure waveforms were also recorded for each test subject in a 37°C water bath. All linear measurements of individual layer thicknesses and total distances were made on-line with use of electronic calipers. A set of multiple and independent insertion loss values, denoted IL_n, was calculated for path *n* between the abdominal surface and the hydrophone from *n* sonograms for each test subject. Each tissue layer type was identified and its thickness along each path *n* was measured. The thickness of tissue type *m* along path *n* was denoted by d_{nm}. The only unknown quantities left were the attenuation coefficients A_m of each of the *m* tissue layers for that test subject. The overestimated set of equations $d_{nm}\ A_m = IL_n$ was solved for A_m with use of a nonnegative least-squares solution technique.

RESULTS: With use of data from 162 independent insertion loss estimate paths, the overall tissue-specific attenuation coefficients for each of the tissue layer types, expressed as mean value ± SD, were 2.3 ± 1.5 dB/cm-MHz for the skin and subcutaneous layer, 3.1 ± 2.5 dB/cm-MHz for skeletal muscle, 0.6 ± 0.5 dB/cm-MHz for myometrium, and 3.6 ± 2.7 dB/cm-MHz for the vaginal wall. The overall insertion loss assuming the "homogeneous" tissue model was 0.7 ± 0.3 dB/cm-MHz.

CONCLUSIONS: We have determined the specific ultrasonic attenuation coefficients for each of the tissue layers comprising the anterior abdominal wall, uterus, and vagina and validated the Food and Drug Administration derating factor of 0.3 dB/cm-MHz applied to obstetric use ultrasonography systems. Of all the models proposed, the "homogeneous" tissue model appears to be the best model for determining ultrasonic exposure risk during reproductive ultrasonographic examinations. (Am J Obstet Gynecol 1999;180:866-74.)

Key words: Ultrasonography, exposimetry, bioeffects, obstetrics and gynecology

Although the clinical use of ultrasonography in the reproductive sciences continues to increase exponentially, having become almost routine in obstetrics,[1] basic scientists have still not resolved the issues of attendant bioeffects and potential biohazards, especially because scientific data establishing the safety of ultrasonography are limited. Experimental observations from animal studies would unequivocally suggest that high-intensity ultrasound has definite and biologically harmful, even lethal, effects.[2] Whether clinical instruments with lower power settings pose similar but less obvious biohazards remains unknown.[3] The issue is further clouded by virtue of minimal in vivo exposimetry data defining actual energy levels to which the ovary or developing embryo and fetus are exposed during the course of a "clinical" ultrasonographic examination.

In a transabdominal obstetric or gynecologic ultrasonographic examination, exposure to ultrasonic energy is attenuated by the intervening abdominal and uterine walls (and placenta in the case of an anteriorly located placenta in an obstetric examination), with the assumption that any intervening fluid medium (amniotic fluid

From the Division of Maternl-Fetal Medicine, Department of Obstetrics and Gynecology, College of Medicine, University of Cincinnati,[a] the Division of Pediatric Cardiology, Children's Hospital Medical Center,[b] and the Department of Electrical Engineering, University of Illinois.[c]

Supported in part by National Institutes of Health grant No. HD 21687.

Presented at the Seventeenth Annual Meeting of The American Gynecological and Obstetrical Society, Hot Springs, Virginia, September 3-5, 1998.

Reprint requests: Tariq A. Siddiqi, MD, Division of Maternal-Fetal Medicine, University of Cincinnati, College of Medicine, PO Box 670526, Cincinnati, OH 45267-0526.

0002-9378/99 $8.00 + 0 ***6/6/97189***

or urine) is essentially lossless. We have constructed an in vivo exposimetry system, developed and tested customized software, and determined selected "first-order ultrasonic quantities" (ie, pressure waveform, peak compressional pressure, peak rarefactional pressure) and selected "second-order ultrasonic quantities" (ie, spatial peak, temporal average intensity, spatial peak, pulse average intensity, spatial peak, temporal peak intensity) during a routine reproductive ultrasonographic examination.[4] We believe that these data are both specific and unique in that they provide the basic scientist with actual in vivo measured ultrasonic exposure levels to test for harmful biologic effects in animal models.

Our previously published in vivo exposimetry data defined the average coefficient of tissue attenuation or insertion loss that occurs during a "routine" reproductive ultrasonographic examination.[5-7] The specific aim of our current investigation was to determine in vivo tissue-specific ultrasonographic attenuation coefficients for each of the tissue layers composing the anterior abdominal wall, uterus, and vagina with use of a quantitative multilayer tissue model. We wanted to combine these data with our previously published human ultrasound exposimetry studies to further validate the "homogeneous" tissue model–based Food and Drug Administration (FDA) derating factor of 0.3 dB/cm-MHz applied to obstetric-use ultrasound systems.

Material and methods

Diagnostic imaging system. A 3.0-MHz frequency mechanical sector transducer (focal zone 5.5 to 13 cm, focal point 8 cm, crystal diameter 19 mm) in combination with an ATL Ultramark 4 model (Advanced Technology Laboratories, Bothell, Wash) diagnostic ultrasound imaging system was used for all studies. Exposure time after each acceptable real-time image was obtained was 2 minutes. The power setting for the instrument was 100% at all times.

Exposimetry instrumentation. The customized exposimetry equipment and software for in vivo studies have been previously reported in detail.[4-7] In summary, instrumentation has been developed to measure the acoustic pressure field during a diagnostic reproductive system ultrasonographic examination. The ultrasonic field is sampled with use of a calibrated 7-element linear-array hydrophone of polyvinylidene difluoride transducers. This hydrophone is placed in the anterior vaginal fornix superior to the cervix. The radio frequency ultrasonic signals from the hydrophone are digitized at 50 megasamples per second and the maximum amplitude in waveform received in the examination is recorded along with that radio frequency waveform. The reference output of the clinical real-time scanner is obtained by placing the hydrophone in a 37°C water bath at the same distance from the clinical transducer as that used to obtain the in vivo recording. From the hydrophone recordings, 10 exposimetry quantities are determined, 5 under in vivo conditions and 5 under in vitro conditions. The 5 quantities are the maximum peak compressional pressure, the maximum peak rarefactional pressure, the spatial peak, temporal average intensity, the spatial peak, pulse average intensity, and the spatial peak temporal peak intensity.[4,5]

Subject population. Healthy nonpregnant female volunteers were recruited for the study. Each subject was counseled and asked to sign an informed consent statement as approved by the University of Cincinnati Medical Center Institutional Review Board. Subjects were studied under 2 conditions: some in the presence of a full urinary bladder where the ultrasound beam traversed the anterior abdominal wall and distended bladder before reaching the location of the hydrophone, whereas others were studied immediately after emptying the urinary bladder where the ultrasound beam traversed the anterior abdominal wall and anteflexed uterus (myometrium)before reaching the location of the hydrophone in the anterior vaginal fornix.

Hydrophone placement and study protocol. Study subjects with a full bladder were asked to force fluids for 1 hour before the planned time of the study. When each subject felt that her urinary bladder was distended and there was an urge to void, she was placed in a supine position with hips abducted and knees flexed and externally rotated (ie, in a "frog-leg" configuration). The custom-designed hydrophone was then introduced into the vagina and placed in the anterior vaginal fornix superior to the cervix. Placement was checked with use of real-time imaging and care was taken to ensure that the urinary bladder was in fact full with complete cephalad displacement of the pelvic organs by the distended bladder. The lower end of the hydrophone has a recognizable round flange to help ensure appropriate placement and orientation of the linear array of transducers. The total distance from the transducer to the hydrophone and the individual thickness of each intervening maternal tissue layer (ie, the skin and subcutaneous tissue, skeletal muscle, urinary bladder [excluding the anterior and posterior bladder walls], myometrium, and vaginal wall) were then measured on-line with use of real-time imaging and electronic calipers. In vivo data were then obtained at 100% power settings for the diagnostic imaging system over a 2-minute period. The largest hydrophone signal was saved for analysis. This protocol was repeated for each subject to obtain the maximum in vivo pulse average intensity values from a minimum of 5 different transducer locations on the abdominal surface and different receiving hydrophone locations. Each transducer location was separated (center-to-center) by twice the distance of the transducer's beam width.

Study subjects with an empty bladder were asked to

empty their urinary bladders immediately before being studied. The same custom-designed hydrophone used previously was inserted into the vagina and placed in the anterior vaginal fornix with slight forward pressure to further flex the anteverted uterus, thereby ensuring closest proximity between the hydrophone and uterus. Subjects with retroverted uteruses were excluded from the study and some subjects who were studied in the full bladder state were also studied in the empty bladder condition. The total distance from the transducer to the hydrophone and the individual thickness of each intervening maternal tissue layer (ie, the skin and subcutaneous tissue, skeletal muscle, myometrium, and vaginal wall) were then measured on-line with use of real-time imaging and electronic calipers. In vivo data were then obtained in the same manner as previously described (ie, from a minimum of 5 different transducer locations on the abdominal surface and different receiving hydrophone locations).

Calibration (in vitro) data for each transducer location were recorded immediately after completion of the in vivo study in water (37°C) and with the transducer-hydrophone distance the same as in the respective in vivo state. Again, the largest signal recorded over a 2-minute period during the in vitro procedure was saved for data analysis.

Data analysis. For each subject (full bladder or empty bladder condition treated as an independent state even in the same individual), a complete data set of in vivo and in vitro pressure waveforms was obtained for each transducer location with corresponding sonograms for tissue-path distance and individual intervening tissue layer thickness measurements. Six insertion loss values (loss as determined by the measurement procedure) were calculated for each transducer location:

$$IL = 20 \log_{10} \left(\frac{\text{In vivo pressure}}{\text{In vitro pressure}} \right) \quad (1)$$

where IL is insertion loss and the pressure ratios were for peak compressional pressure, peak refractional pressure, and peak compressional pressure plus peak refractional pressure, and

$$IL = 10 \log_{10} \left(\frac{\text{In vivo intensity}}{\text{In vitro intensity}} \right) \quad (2)$$

where the intensity ratios were for temporal average intensity, pulse average intensity, and temporal peak intensity. A mean insertion loss value of the 6 insertion loss values represented the insertion loss value for each transducer location for each subject for subsequent calculations.

When possible, there was a minimum of twice the number of abdominal transducer positions as the number of tissue layers to obtain an appropriate overestimated set of equations. The multiple and independent insertion loss values, denoted by IL_n, represent the estimated insertion loss of path n (number of transducer positions) between the abdominal surface and the hydrophone. From the n sonograms identification of tissue layer types and distances within those tissue layers along each path n were made and entered into the computer file of that test subject. The distance through tissue type m (number of tissue layers) along path n is denoted by d_{nm}. The only unknown quantities were the attenuation coefficient (A_m) values of each of the m tissue layers. Therefore the overestimated set of equations

$$d_{nm}A_m = IL_n \quad (3)$$

was solved for A_m for each subject evaluated.

For each test subject the acquired (in vivo) and calibration (in vitro) waveforms were loaded into MATLAB (The Math Works, Natick, Mass) on a SUN (Palo Alto, Calif) workstation and analyzed independently of the data acquisition system. Pressure and intensity measures and their respective insertion loss values were calculated and compared with the results generated by the data acquisition system to verify data integrity. In addition, the layer thickness information was loaded into the system. A nonnegative least-squares solution was calculated for the overdetermined system of equations, as defined by equation 3. For each test subject individual layer attenuation coefficient values were then estimated and recorded.

Two types of analyses were performed from the upgraded exposimetry system, which provided the capability to acquire multiple paths between the maternal abdominal surface and the in situ hydrophone location.

The first was an estimation of the attenuation coefficient for each of the intervening tissue layers between the maternal abdominal surface and the in situ hydrophone location with use of the method described above.

The second was an estimation of the various published tissue models, fixed-path *(fp),* homogeneous *(ho),* and overlying *(ov).*[4-11] These respective attenuation coefficients for each subject were determined by the equations

$$A_{fp} = \frac{\text{Mean IL}}{f_c} \quad (4)$$

$$A_{ho} = \frac{\text{Mean IL}}{d_{total} f_c} \quad (5)$$

$$A_{ov} = \frac{\text{Mean IL}}{d_{ov} f_c} \quad (6)$$

where IL is insertion loss value, f_c is the center frequency in megahertz, and d_{total} and d_{ov} are, respectively, the total distance between the skin surface and the hydrophone and tissue thickness of the overlying intact tissue between the skin surface and hydrophone, that is,

$$d_{total} = d_{abd\ wall} + d_{bladder} + d_{uterus} + d_{vaginal\ wall} \quad (7)$$

$$d_{ov} = d_{total} - d_{bladder} \quad (8)$$

where $d_{abd\ wall}$, $d_{bladder}$, d_{uterus}, and $d_{vaginal\ wall}$ are the individual thicknesses for the abdominal wall, bladder

Table I. Individual tissue layer thicknesses for empty-bladder (n = 49 independent insertion loss estimate paths from 9 subjects) and full-bladder (n = 135 independent insertion loss estimate paths from 23 subjects) conditions (mean value ± SD)

	Empty bladder (n = 49)	*Full bladder (n = 135)*	*Empty vs full bladder (statistical significance)**	*Combined (n = 184)*
Skin and subcutaneous tissue (cm)	1.3 ± 0.5	1.6 ± 0.7	*P* = .005	1.5 ± 0.6
Skeletal muscle (cm)	1.1 ± 0.6	1.1 ± 0.3	*P* = .15	1.1 ± 0.4
Urinary bladder (cm)	†	5.3 ± 1.0		
Myometrium (cm)	4.7 ± 1.0	‡		
Vaginal wall (cm)	0.5 ± 0.3	0.6 ± 0.2	*P* = .034	0.6 ± 0.3

*Mann Whitney *U* test.
†Urinary bladder was not within ultrasound path for empty-bladder condition.
‡Myometrium was not within ultrasound path for the full-bladder condition.

Table II. Attenuation coefficient values in decibels/centimeters-megahertz for indicated tissue layers for empty-bladder (n = 5 subjects) and full-bladder (n = 21 subjects) conditions (mean value ± SD)

	Empty bladder (n = 5)	*Full bladder (n =21)*	*Empty vs full bladder (statistical significance)**	*Combined (n = 26)*
Skin and subcutaneous tissue	2.7 ± 1.0	2.2 ± 1.5	*P* = .48	2.3 ± 1.5
Skeletal muscle	2.0 ± 0.8	3.3 ± 2.7	*P* = .91	3.1 ± 2.5
Myometrium	0.6 ± 0.5	†		
Vaginal wall	2.9 ± 2.1	3.7 ± 2.8	*P* = .83	3.6 ± 2.7

*Mann Whitney *U* test.
†Myometrium was not within ultrasound path for full-bladder condition.

Table III. Measured and calculated quantities for empty-bladder (n = 42 independent insertion loss estimate paths from 9 subjects) and full-bladder (n = 120 independent insertion loss estimate paths from 23 subjects) conditions (mean value ± SD)

	Empty bladder (n = 42)	*Full bladder (n = 120)*	*Empty vs full bladder (statistical significance)**	*Combined (n = 162)*
Mean $d_{abd\ wall}$ (cm)	2.5 ± 0.9	2.6 ± 0.9	*P* = .12	2.6 ± 0.9
Mean $d_{bladder}$ (cm)	†	5.4 ± 1.0		
Mean $d_{myometrium}$ (cm)	4.7 ± 1.0	‡		
Mean d_{ov} (cm)	7.7 ± 1.3	3.3 ± 0.9	*P* < .0001	4.4 ± 2.2
Mean d_{total} (cm)	7.7 ± 1.3	8.6 ± 1.3	*P* = .0014	8.4 ± 1.3
Mean insertion loss (dB)	17.3 ± 5.4	13.4 ± 4.7	*P* < .0001	14.4 ± 5.1
Mean A_{fp} (dB/MHz)	7.2 ± 2.2	5.6 ± 2.0	*P* < .0001	6.0 ± 2.1
Mean A_{ho} (dB/cm-MHz)	1.0 ± 0.4	0.7 ± 0.3	*P* < .0001	0.7 ± 0.3
Mean A_{ov} (dB/cm-MHz)	1.0 ± 0.4	1.8 ± 0.8	*P* < .0001	1.6 ± 0.8

*Mann-Whitney *U* test.
†Urinary bladder was not within ultrasound path for empty-bladder condition.
‡Myometrium was not within the ultrasound path for full-bladder condition.

($d_{bladder}$ assumed 0 for empty bladder group), uterus, and vaginal wall, respectively. The individual thickness measurements were made on-line with use of electronic calipers at the time of the ultrasonographic examination.

Statistical methods. The 2-tailed unpaired Mann-Whitney *U* test was used to compare the medians of the 2 unpaired groups because the SDs of the 2 groups could not be assumed equal, a requirement of the *t* test that compares the means of 2 groups. Linear regression analysis was used to quantify the best-fit straight line between 2 variables. The slope's *P* value indicates the slope's significance relative to a 0 slope and the adjusted sample coefficient of determination (adjusted r^2) accounts for the variation in the dependent variable. The adjusted r^2 is the sample coefficient of determination (r^2) adjusted for degrees of freedom.[12] The ±90% confidence intervals of the mean of the dependent variable are provided when the regression equation is shown graphically. Multiple regression analysis was used to quantify the best-fit estimate between more than 2 variables and the *P* values are determined for each of the estimated coefficients to test their significant differences from 0. Statistical sig-

Table IV. Measured and calculated quantities for empty-bladder (42 independent insertion loss estimate paths from 9 subjects) and full-bladder (120 independent insertion loss estimate paths from 23 subjects) conditions from this study (see Table III) combined with similar quantities for empty-bladder (48 independent insertion loss estimate paths from 48 subjects) and full-bladder (41 independent insertion loss estimate paths from 41 subjects) conditions from 2 previous studies[4, 5] for a total of n = 90 independent insertion loss estimate paths from 57 subjects for empty-bladder condition and n = 161 independent insertion loss estimate paths from 64 subjects for full-bladder condition (mean value ± SD)

	Empty bladder (n = 90)	*Full bladder (n = 161)*	*Empty vs full bladder (statistical significance)**	*Combined (n = 251)*
Mean $d_{abd\ wall}$ (cm)	3.1 ± 1.4	2.7 ± 0.9	*P* = .11	2.8 ± 1.1
Mean $d_{bladder}$ (cm)	†	5.3 ± 1.0		
Mean $d_{myometrium}$ (cm)	4.2 ± 1.5	‡		
Mean d_{ov} (cm)	7.5 ± 1.5	3.1 ± 0.9	*P* < .0001	4.7 ± 2.4
Mean d_{total} (cm)	7.5 ± 1.5	8.4 ± 1.3	*P* < .0001	8.1 ± 1.5
Mean insertion loss (dB)	13.7 ± 6.8	11.9 ± 5.4	*P* = .039	12.5 ± 6.0
Mean A_{fp} (dB/MHz)	5.7 ± 2.8	4.9 ± 2.3	*P* = .039	5.2 ± 2.5
Mean A_{ho} (dB/cm-MHz)	0.8 ± 0.4	0.6 ± 0.3	*P* = .0005	0.7 ± 0.3
Mean A_{ov} (dB/cm-MHz)	0.8 ± 0.4	1.7 ± 0.8	*P* < .0001	1.3 ± 0.8

*Mann-Whitney *U* test.
†Urinary bladder was not within ultrasound path for empty-bladder condition.
‡Myometrium was not within ultrasound path for full-bladder condition.

nificance is assumed at the .05 level and statistical calculations were performed with use of Excel 5.0 (Microsoft, Inc, Redmond, Wash) and Instat 2.00 (GraphPad Software, San Diego, Calif).

Results

A total of 34 nonpregnant subjects were studied with use of the quantitative multilayer data acquisition exposimetry technique, 25 in the full-bladder and 9 in the empty-bladder state. The acquired data from 2 of the full-bladder subjects were corrupted and could not be used. Also, the acquired radio frequency signal under both in vivo and in vitro conditions yielded a center frequency of 2.4 MHz, which was used in all calculations.

Attenuation coefficient of each intervening tissue layer. The attenuation coefficient for each of the intervening tissue layers was estimated by use of the measured tissue thicknesses and the calculated insertion loss values. Table I lists the intervening tissue thicknesses for 49 independent insertion loss estimate paths from the 9 empty-bladder subjects and 135 independent insertion loss estimate paths from the 23 full-bladder subjects.

On the basis of our data rejection criteria to provide the best estimates of insertion loss for estimating attenuation coefficients of the intervening maternal tissue layers, 7 insertion loss estimates were rejected from the empty-bladder database and 15 from the full-bladder database. This yielded a total of 42 independent insertion loss estimates for the 9 empty-bladder subjects and 120 insertion loss estimates for the 23 full-bladder conditions.

The least-squares solution did not provide stable solutions for 4 of the empty-bladder cases and 2 of the full-bladder cases. The principal reason for this appears to be the lack of well-defined independent equations as a result of similar individual layer distances, d_{nm}, from each of the independent paths, *n*. The estimated attenuation coefficients of each intervening maternal tissue layer—skin and subcutaneous tissue, skeletal muscle, myometrium, and vaginal wall—are listed in Table II. The bladder's fluid for the full-bladder cases was assumed to be lossless.

Estimation of various published tissue models (fixed-path, homogeneous, and overlying). The intervening tissue distance, mean insertion loss, and the attenuation coefficient (from equations 4 to 6) results for the 42 independent insertion loss estimates from the 9 empty-bladder subjects and 120 insertion loss estimates from the 23 full-bladder subjects are described in the form used in Table III to provide the basis to compare the results of our currently acquired data with those reported previously. In our previous investigation we estimated these ultrasonic quantities under human ovary exposure conditions during a transabdominal ovarian ultrasonographic examination and published these data for 43 independent insertion loss estimates, 18 in the full-bladder and 25 in the empty-bladder state.[5] To these data, obtained with improved exposimetry equipment and software, we have added data from an additional 46 independent insertion loss estimates, 23 in the full-bladder and 23 in the empty-bladder state.[6] Table IV summarizes the results for all 251 experiments in nonpregnant subjects (90 with empty bladders and 161 with full bladders); it should be noted that the results are based on independent insertion loss data acquisitions wherein there was only 1 independent insertion loss data acquisition per experiment in our 2 previous reports.

Comment

Classically, the anatomic layers comprising the anterior abdominal wall are skin, superficial fascia (Camper's fatty and Scarpa's membranous layers), 3 flat muscles (external oblique, internal oblique, and transversus) and their fascia, the paired rectus abdominus muscles and enveloping fascia in the midline, preperitoneal fatty areolar tissue, and peritoneum. It is, however, not possible to separately distinguish each of these tissue types forming the anterior abdominal wall during clinical ultrasonography, so they are therefore grouped into the 2 tissue-layer categories defined in our study as (1) skin and subcutaneous tissue and (2) skeletal muscle, which includes the investing fascial layers, preperitoneal fat, and peritoneum.

Skin consists of 2 layers, the epidermis and dermis, with an average total thickness of approximately 2 mm in a nonspecialized location such as the abdomen. The epidermis is composed of cornified squamous epithelium, whereas the dermis is a layer of connective tissue that is more densely packed and together with its constituent fine nerves, blood vessels, glands, specialized cells, and matrix forms a base for the epidermis. The attenuation coefficient for skin is estimated to be between 1 and 4 dB/cm-MHz in the 1- to 10-MHz frequency range.[13, 14] On its deep surface the dermis is connected to the superficial fascia (tela subcutanea). This layer, also known as the subcutaneous layer, is made up of loose connective tissue (areolar tissue) and Camper's fatty and Scarpa's membranous layers. Areolar tissue has a fine, cobweb-like structure and is formed by a framework of interwoven collagen fibers intermingled with occasional elastic fibers filled with fluid and specialized cells and may, as over the abdomen, contain a large number of fat cells (Camper's layer). The underlying Scarpa's layer is a more readily anatomically distinguishable membranous layer composed of denser tissue (greater content of collagen fibers) that lies superficial to the investing fascial layer of the flat muscles and rectus abdominus. The thickness of the subcutaneous layer is variable and may be associated with overall body weight. The attenuation coefficient for fat is estimated to be between 1.1 and 2.1 dB/cm-MHz in the 2 to 7 MHz frequency range[13, 15] and that for connective tissue is estimated to be as high as 5 dB/cm-MHz in this frequency range.[16] In our current investigation the combined mean ± SD attenuation coefficient value for the skin and subcutaneous layer was 2.3 ± 1.5 dB/cm-MHz. Although these data are from nonpregnant healthy volunteers, our average thickness for the combined skin and subcutaneous layers (1.5 ± 0.6 cm) is not much different from the mean value of 1.6 cm reported by Carson et al,[8] whereas it is almost twice that of 0.83 cm reported by Ramnarine et al[17] and 1.09 cm reported by Kamel[18] for pregnant women.

The next intervening layer, comprising skeletal muscles of the anterior abdominal wall with their investing fascia, is also of variable thickness with the attenuation coefficient of skeletal muscle estimated to be between 0.9 and 3.1 dB/cm-MHz, depending on orientation between the ultrasound beam and tissue fibers, in the 1 to 10 MHz frequency range.[13, 19, 20] Between this muscle layer and the peritoneum, which comprises a layer of elastic areolar tissue lined on both sides by mesothelial cells, there may be a layer of fatty areolar tissue referred to as the preperitoneal fat layer. Our experimentally derived ultrasonic attenuation coefficient value for skeletal muscle was 3.1 ± 2.5 dB/cm-MHz. Similarly, our mean value for the skeletal muscle layer thickness (including fascia, preperitoneal fat, and the anterior wall of the urinary bladder in the full-bladder condition, 1.1 ± 0.4 cm) is greater than the previously reported value of 0.6 cm by Carson et al[8] and 0.52 cm by Ramnarine et al[17] by a factor of 2, whereas it is similar to the value of 0.91 cm reported by Kamel.[18] Most of these differences can probably be attributed to the fact that we are comparing the nonpregnant with the pregnant state and even within the pregnant data the measurements are spread across all 3 trimesters.

In the current study of nonpregnant women we also wanted to determine the influence, if any, of an interposing urinary bladder (fluid medium) on the overall insertion loss characteristics of the various tissue layers. Urine was assumed, as previously stated, to be lossless so that the anterior wall of the urinary bladder was not separately distinguished and is included in the skeletal muscle thickness measurement, whereas the posterior wall of the urinary bladder is included in the vaginal wall thickness measurements in the full-bladder condition. This explains why the anterior abdominal and vaginal walls measure thicker in the full-bladder condition (Table II). In the presence of an anteflexed uterus (all cases studied), the empty bladder was not within the ultrasound path.

The attenuation coefficient for myometrium is estimated to be between 0.23 and 1.9 dB/cm-MHz in the 1- to 10-MHz frequency range,[13] whereas we have before this study estimated it to be 0.14 dB/cm-MHz for the pregnant uterus.[6] Data relative to an expected attenua-

Table V. Constituent composition of various tissue layers of interest in gynecologic ultrasonographic examination (modified from Duck[16])

	Water (%)	*Lipid (%)*	*Ash (%)*	*Protein (%)*
Skin	65.3	9.4	0.3	4.4
Fat	21.2	71.4	0.3	4.4
Skeletal muscle	74.1	4.2	1.0	19.8
Fascia	70.0	1.9	1.4	27.0
Myometrium	79.0	1.4	1.0	20.0

tion coefficient for the vaginal wall do not exist to our knowledge. In our current investigation the attenuation coefficient was 0.6 ± 0.5 dB/cm-MHz for myometrium and 3.6 ± 2.7 dB/cm-MHz for the vaginal wall. The nonpregnant myometrial thickness obviously cannot be compared with the myometrial thickness in the gravid state, whereas anatomically the vaginal wall is approximately 5 mm thick, which is consistent with our ultrasonographically derived measurement.

Overall, our estimated tissue-specific mean attenuation coefficient values are generally greater than the mean attenuation coefficient values that have traditionally been ascribed to these tissues, which are probably an underestimate.[8, 13] The general trend is encouraging, although, as mentioned, the nonnegative least-squares solution technique yields estimates greater than should be. Additionally, our data are for the nonpregnant state, where the water retention in all tissues that occurs during pregnancy is not factored in and would induce a generalized decrease in the attenuation coefficient of all tissues.[21]

The constituent composition of the tissues of interest in terms of water, lipid, ash, and protein content is shown in Table V.[16] As may be noted, other than for fat, the remaining tissue types are quite similar in their overall water and protein content. Because skin and fat are included as a single layer in our data, the mean attenuation value is somewhat lower than that for skeletal muscle and the vaginal wall, which is essentially a musculofascial tube and is best approximated by fascia as a tissue type. The uterus has a low attenuation coefficient generally because even in the nonpregnant state it is a very vascular organ and whole blood has a relatively low attenuation coefficient of about 0.02 dB/cm-MHz at 2.4 MHz.[22] From our previous human in situ differential insertion loss results[6] we estimated the mean tissue attenuation coefficients to be 1.39 dB/cm-MHz for the abdominal wall and 0.14 dB/cm-MHz for the myometrium, suggesting that the abdominal wall is the principal source of ultrasonic energy loss in a transabdominal reproductive ultrasonographic examination. Consistent with these earlier results, the myometrium's attenuation coefficient is considerably lower (0.6 ± 0.5 dB/cm-MHz) than that of the abdominal wall (skin and subcutaneous tissue layer and skeletal muscle, 2.3 ± 1.5 dB/cm-MHz and 3.1 ± 2.5 dB/cm-MHz, respectively).

We have previously determined the maximum in vivo values of ultrasonic quantities during a transabdominal gynecologic ultrasonographic examination. We reported these data[5] for 43 experiments in nonpregnant women (18 with full bladders and 25 with empty bladders). We have continued these studies with the improved exposimetry equipment and software with an additional 46 experiments in nonpregnant test subjects (23 with full bladders and 23 with empty bladders).[6] Our most recent data combined with our previous nonpregnant database is shown in Table IV, yielding a much more complete nonpregnant database of 121 experiments (57 with empty bladders and 64 with full bladders). The earlier nonpregnant examination results exhibited similar total distances between the maternal abdominal wall and the hydrophone (ie, in the range of 7 cm). However, the more recent data exhibited a greater insertion loss.

A number of tissue models have been proposed to estimate the in vivo exposure levels from measurements of acoustic output made in water to have an improved basis for estimating fetal risk during an obstetric ultrasonographic examination. The "fixed-path" tissue model is based on the assumptions that the ultrasonic attenuation between the skin surface and conceptus is linearly dependent on frequency and independent of distance.[5, 7-9] The "homogeneous" tissue model is based on the assumption that the ultrasonic attenuation occurs uniformly over the total distance between the skin surface and the conceptus.[7, 9-11] The "overlying" tissue model is based on the assumptions that the ultrasonic attenuation occurs uniformly within intact tissue only and that there is negligible attenuation from any intervening fluid path.[4-7]

The homogeneous attenuation coefficient for both full- and empty-bladder conditions exhibit quite similar results; the combined value is 0.7 ± 0.3 dB/cm-MHz, which is only greater than the value of 0.3 dB/cm-MHz used for regulatory purposes by the FDA[10, 11, 23] and the value of 0.44 dB/cm-MHz recommended for use by the National Council on Radiation Protection and Measurements.[9] This would suggest that the FDA derating factor of 0.3 dB/cm-MHz continues to be appropriate assuming, of course, the purpose of the FDA derating factor is to overestimate the exposure level in reproductive examinations (worst-case situation), thereby ensuring that the fetus is not exposed to biologically harmful ultrasonic exposure levels. The fixed-path attenuation coefficient values between 0.5 and 1 dB/MHz recommended for various stages of pregnancy by the National Council on Radiation Protection and Measurements[9] may not be appropriate compared with our mean value of 5.2 ± 2.5 dB/MHz, although our results were acquired from nonpregnant test subjects. The overlying attenuation coefficient does not appear to be appropriate at all for estimating in utero exposure levels because there is about a factor of 2 difference between its empty-bladder versus full-bladder values.

REFERENCES

1. Carrera JM, Torrents M, Mortera C, Cusi V, Muñoz A. Routine prenatal ultrasound screening for fetal abnormalities: 22 years' experience. Ultrasound Obstet Gynecol 1995;5:174-9.
2. O'Brien WD Jr. Ultrasound bioeffect issues related to obstetric sonography and related issues of the output display standard. In: Fleischer AC, Manning FA, Jeanty P, Romero R, editors. The principles and practice of ultrasonography in obstetrics and gynecology. 5th ed. Norwalk (CT): Appleton & Lange; 1995. p. 17-33.
3. Fischer JE Jr, Acuff-Smith KM, Schilling A, Meyer RA, Smith NB, Moran MS, et al. Behavioral teratologic evaluation of prenatal

exposure to pulsed-wave ultrasound in unanesthetized rats. Teratology 1996;54:65-72.
4. Daft CMW, Siddiqi TA, Fitting DW, Meyer RA, O'Brien WD Jr. In-vivo fetal exposimetry. IEEE Trans Ultrasonics Ferroelectrics Frequency Cont 1990;37:500-5.
5. Siddiqi TA, O'Brien WD Jr, Meyer RA, Sullivan JM, Miodovnik M. In situ exposimetry: the ovarian ultrasound examination. Ultrasound Med Biol 1991;17:257-63.
6. Siddiqi TA, O'Brien WD Jr, Meyer RA, Sullivan JM, Miodovnik M. Human in situ dosimetry: differential insertion loss during passage through abdominal wall and myometrium. Ultrasound Med Biol 1992;18:681-9.
7. Siddiqi TA, O'Brien WD Jr, Meyer RA, Sullivan JM, Miodovnik M. In situ human obstetrical ultrasound exposimetry: estimates of derating factors for each of three different tissue models. Ultrasound Med Biol 1995;21:379-91.
8. Carson PL, Rubin JN, Chiang EH. Fetal depth and ultrasound path lengths through overlying tissues. Ultrasound Med Biol 1989;15:629-39.
9. National Council on Radiation Protection and Measurements. Exposure criteria for medical diagnostic ultrasound. I. Criteria based on thermal mechanisms. National Council on Radiation Protection and Measurements. Bethesda: The Council; 1992. Report No.: 113.
10. Food and Drug Administration. 501(k) Guide for measuring and reporting acoustic output of diagnostic ultrasound medical devices, December, 1985. Rockville (MD): Center for Devices and Radiological Health, US Food and Drug Administration; 1985.
11. Food and Drug Administration. Revised 510(k) diagnostic ultrasound guidance for 1993. Rockville (MD): Center for Devices and Radiological Health, US Food and Drug Administration; 1993.
12. Hamburg M. Basic Statistics: a modern approach. New York: Harcourt Brace Jovanovich; 1979.
13. National Council on Radiation Protection and Measurements. Biological effects of ultrasound: mechanisms and clinical implications. Bethesda (MD): National Council on Radiation Protection and Measurements; 1983. Report No.: 74.
14. Riederer-Henderson MA, Olerud JE, O'Brien WD Jr, Forster FK, Steiger DL, Ketterer DJ, et al. Biochemical and acoustical parameters of normal canine skin. IEEE Trans Biomed Eng 1988;35:967-72.
15. Haney MJ, O'Brien WD Jr. Temperature dependency of ultrasonic propagation properties in biological materials. In: Greenleaf JF, editor. Volume 1: tissue characterization with ultrasound. Boca Raton (FL): CRC Press; 1986. p. 15-55.
16. Duck FA. Physical properties of tissue: a comprehensive reference book. New York: Academic Press; 1990.
17. Ramnarine KV, Nassiri DK, Pearce JM, Joseph AEA, Patel RH, Varma TR. Estimation of in situ ultrasound exposure during obstetric examinations. Ultrasound Med Biol 1993;19:319-29.
18. Kamel IR. An evaluation of overlying tissues to determine fetal exposure to ultrasound during the third trimester. Ultrasound Med Biol 1994;20:42-51.
19. Roberjot V, Laugier P, Berger G. Anisotropy in bovine skeletal muscle in vitro: frequency dependent attenuation and backscatter coefficient over a wide range of frequencies. In: Proceedings of the Ultrasonics Symposium; 1994 Nov 1-4; Cannes, France. New York: IEEE; 1994. p. 1467-70.
20. Smith NB. Effect of myofibril length and tissue constituents on acoustic propagation properties of muscle [dissertation]. Champaign (IL): University of Illinois at Urbana-Champaign; 1996.
21. Pipe NGJ, Smith T, Halliday D, Edmonds CJ, Williams C, Coltart TM. Changes in fat-free mass and body water in human normal pregnancy. Br J Obstet Gynaecol 1979;86:929-40.
22. Carstensen EL, Li K, Schwan HP. Determination of the acoustic properties of blood and its components. J Acoust Soc Amer 1953;25:286-9.
23. Food and Drug Administration. Information for manufacturers seeking marketing clearance of diagnostic ultrasound systems and transducers. Rockville (MD): Center for Devices and Radiological Health, US Food and Drug Administration; 1997.

Discussion

Dr Rudy E. Sabbagha, Chicago, Illinois. In this study the authors investigated the attenuation of an ultrasound beam through each of the tissue layers of the abdominal wall in nonpregnant patients. The degree of attenuation was obtained in full- and empty-bladder conditions. The insertion loss was determined by a standard formula comparing in vivo attenuation of pressure waveforms with those in a lossless water medium, simulating the exact in vivo conditions. The authors described 4 layers that the ultrasound beam could traverse. These layers are skin and subcutaneous tissue, skeletal muscle, myometrium, and vaginal wall. In patients with a full bladder, a design that actually corresponds to the clinical examination, the greatest attenuation resulted from the vaginal wall (3.7 ± 2.8 dB/cm-MHz) and was followed by skeletal muscle (3.3 ± 2.7 dB/cm-MHz), skin and subcutaneous tissue (2.2 ± 1.5 dB/cm-MHz), and myometrium or vaginal wall (0.23 to 1.9 dB/cm-MHz). By contrast, in the empty-bladder arm of the experiment the greatest attenuation was from the skin and subcutaneous tissue.

Subsequently, the authors examined the attenuation coefficients in relation to 3 established tissue models. They found that the homogenous tissue model exhibited results similar to those of the study. The homogeneous model is based on the assumption that attenuation occurs uniformly over the total distance between the skin surface and hydrophone. The homogeneous attenuation coefficient in subjects with full- or empty-bladder conditions was 0.7 ± 0.3 dB/cm-MHz. By comparison, Siddiqi et al have previously shown that the attenuation coefficient of the human pregnant uterus is much lower or 0.14 dB/cm-MHz,[1] a finding attributed to the increased vascularity and water retention in association with pregnancy. It is clear then that the attenuation coefficient in the nonpregnant state is greater than that during pregnancy and comparing one with the other would not be justified. Even the FDA attenuation coefficient of 0.3 dB/cm-MHz, an industry regulatory standard, is greater than the value of 0.14 dB/cm-MHz reported by Siddiqi et al.[1]

Let me place these data in more perspective. Although exposimetry remains important, the "rules" have changed.[2] Recently the FDA increased the maximum allowable output of the spatial peak and temporal average intensity from 94 mW/cm^2 to 720 mW/cm^2, but the FDA also required that the thermal index and the mechanical index be displayed on the screen.[3]

These 2 indexes are a direct reflection of tissue heat and inertial cavitation, the 2 mechanisms by which ultrasound induces biologic effects in tissues. Separate algorithms are used to calculate these indexes. The thermal index depends on the product of the temporal average intensity (in both the "on" and "off" cycles of the pulsed wave) and the absorption coefficient (which increases linearly with increasing frequency). The mechanical index depends on the highest intensity level or temporal peak intensity and the frequency of the transducer used.

The thermal index displays the anticipated temperature rise of the insunated tissue, in degrees Celsius, within the focal area of the ultrasound beam. It is not

clear at this time whether thermal teratogenic effects occur by a given elevation of the temperature over the normal level or by the absolute temperature reached. Nonetheless, no biologic ill effects are expected when the increase in body temperature does not exceed 1°C.

A mechanical index of ≥0.5 assumes that inertial cavitation may occur, in the form of either vibration of gaseous bubbles or violent bubble collapse. These events produce free radicals and hydrogen peroxide, chemicals implicated in inducing mutations or single-strand breaks in solutions of deoxyribonucleic acid. Of importance is that, unlike the adult, the fetal lung and gastrointestinal tract are devoid of gas bubbles. As a result, the potential for inertial cavitation in the fetus is reduced. Pulse echo contrast agents are known to introduce gas bubbles into patients but they are not used in obstetrics.

In summary, the authors of this article have shown us the complexity involved in studying the in vivo attenuation of ultrasound; they demonstrated that the attenuation is greater in the gynecologic patient than it is during pregnancy. In the future, the biologic effects of ultrasound, in the reproductive set up, have to be studied within the context of the thermal index and the mechanical index, incorporating the new maximum intensity output of 720 mW/cm^2.

I have 3 questions. (1) How do you explain the low attenuation coefficient of the skin and subcutaneous tissue in the full-bladder condition? (2) Since the homegeneous tissue model exhibited results similar to those of your study, can we assume that it would no longer be necessary to examine the attenuation of each tissue layer separately? (3) Because high-frequency transvaginal transducers are now relied on to evaluate the early pregnancy and ovarian follicles, what is your insight regarding the applicability of the transabdominally derived attenuation coefficients to current imaging methods?

REFERENCES

1. Siddiqi TA, O'Brien WD Jr, Meyer RA, Sullivan JM, Miodovnik M. Human in situ dosimetry: differential insertion loss during passage through abdominal wall and myometrium. Ultrasound Med Biol 1992;18:681-9.
2. Miller MW, Brayman AA, Abramowicz JS. Obstetric ultrasonography: a biophysical consideration of patient safety—the "rules" have changed. Am J Obstet Gynecol 1998;179:241-54.
3. Center for Devices and Radiologic Health. Use of mechanical index in place of spatial peak, pulse average intensity in determining substantial equivalence. Rockville (MD): Food and Drug Administration; 1994.

Dr Jacob Rotmensch, Chicago, Illinois. The attenuation factor relies on the coefficient of density. What was the coefficient of density of the various layers, the skin to fat and muscle?

Dr Steven G. Gabbe, Seattle, Washington. Could you define separately the attenuation coefficients related to skin and subcutaneous tissue, because subcutaneous tissue is probably the greater contributor to this?

Dr Richard L. Berkowitz, New York, New York. I underscore Dr Sabbagha's last question and ask specifically whether this system has been tested with 7-MHz transducers rather than the 3-MHz transducers that were used in this study, because those are what are used commonly in first-trimester scans, where the greatest concern about an effect on a developing embryo has to be considered.

Dr Siddiqi (Closing). Dr Sabbagha asked how we explain the lower attenuation coefficient of the skin and subcutaneous tissue in the full-bladder condition. From a statistical perspective we did not find a significant difference, although the values are, in fact, different. Also, the sample sizes in one condition versus the other are unequal and I accept the criticism, although these are difficult-to-obtain data and the measurement systems are being developed and improved as we conduct this research.

Dr Sabbagha's second question was, because the homogeneous tissue model exhibited results similar to those of our study, can we assume that it will no longer be necessary to examine the attenuation of each tissue layer separately? I don't believe that the research is completely done at this time. I believe this work needs to be further refined, and even though our data support the homogeneous tissue model as the best model to date, we need a much larger database than is available to be able to state absolutely that the homogeneous tissue model can be applied to all situations.

The third question related to high-frequency transvaginal transducers, which are now relied on to evaluate early pregnancy and ovarian follicles and whether transabdominally derived attenuation coefficients can be applied to current imaging methods. At present one can apply the homogeneous tissue model as the best available. If one knows the thickness of the intervening tissue layer, one can determine the exposure level.

I would raise one additional concern that was discussed by Dr Sabbagha with regard to the mechanical index and the potential for cavitation nuclei. Transvaginal ultrasonography, especially when it is used to image ovarian follicles and aspirate ova, introduces gas bubbles; whether these gas bubbles can act as cavitational nuclei and cause damage remains uncertain. These issues have become of much more concern now that the I_{spts} limit has been increased by the FDA from the 94-mW/cm^2 level to 720 mW/cm^2 for obstetric ultrasound systems.

The first question from the floor was regarding the coefficient of density of the tissue exposed to ultrasound. I don't have an answer to that because we did not evaluate tissue in that way. There are data available that deal with the protein, lipid, carbohydrate, and fluid content of various tissues, and we did provide this information in the manuscript.

Dr Gabbe asked about the skin versus the subcutaneous tissue attenuation coefficients. We actually lumped skin and subcutaneous tissue together, because we are not able to evaluate the skin separately because it is so thin.

Finally, Dr Berkowitz asked about 7-MHz versus 3-MHz transducers. We have not checked our system with the 7-MHz frequency transducer.