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## LUMBAR ANNULUS FIBROSUS BIOMECHANICAL CHARACTERIZATION IN CHILDREN BY ULTRASOUND SHEAR WAVE ELASTOGRAPHY

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### Abstract

**Objectives** Intervertebral disc (IVD) is key to spine biomechanics, and it is often involved in the cascade leading to spinal deformities such as idiopathic scoliosis, especially during the growth spurt. Recent progress in elastographic techniques allowed access to noninvasively measure cervical IVD in adults; the aim of this study was to determine the feasibility and reliability of shear wave elastography in healthy children lumbar IVD.

**Methods** Elastographic measurements were performed in thirty-one healthy children (6 to 17 years old), in the annulus fibrosus and in the transverse plane of L5-S1 or L4-L5 IVD. Reliability was determined by 3 experienced operators repeating measurements.

**Results** Average shear wave speed in IVD was  $2.9 \pm 0.5$  m/s; no significant correlations were observed with sex, age or body morphology. Intra-operator repeatability was 5.0 % while inter-operator reproducibility was 6.2 %. Intraclass correlation coefficient was higher than 0.9 for each operator.

**Conclusions** Feasibility and reliability of IVD shear wave elastography was demonstrated. The measurement protocol is compatible with the clinical routine, and the results show the potential to give an insight into spine deformity progression and early detection.

**Keywords:** Spine; Spinal diseases; Fibrocartilage; Tissue elasticity imaging; Pediatrics

### Key points:

- Intervertebral disc mechanical properties are key to spine biomechanics.
- Feasibility of shear wave elastography in children lumbar disc was assessed.
- Measurement was fast and reliable.
- Elastography could represent a novel biomarker for spine pathologies.

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## Introduction

Intervertebral disc (IVD) is a key component of spine biomechanics. It consists of a gelatinous central zone, the *nucleus pulposus*, surrounded radially by the fibro-cartilaginous layers of the *annulus fibrosus* and confined below and atop by the cartilage endplates of the adjacent vertebral bodies [1]. This highly specialized structure allows the spine to be flexible while withstanding high compressive, torsional and shearing loads [2].

IVD has been often suspected to initiate the development and facilitate progression of spine deformities, although it is not necessarily the only one initiating cause. Spinal deformities such as Adolescent idiopathic scoliosis (AIS) are associated with alterations of IVD metabolism, content and morphology [3-6], especially in the lumbar region. The biomechanical environment can affect all these aspects; for instance, according to the Hueter-Volkman principle, the disc-mediated vertebral loading affects the vertebral growth and wedging [7]. This is confirmed by the well-known association between the patient's spinal growth spurt and the onset and progression of AIS.

In normal conditions, with increasing age, water is lost from the matrix and the proteoglycan content changes and diminishes. The disc - particularly the nucleus - becomes less gelatinous and more fibrous, and cracks and fissures eventually form. In AIS patients, minor histological alterations of the disc can be observed as soon as 3-7 years of age and they significantly increase after 16 years of age [8]; asymmetric loading, which is characteristic of scoliosis, can add to the detrimental effects of aging on disc biology [9].

The role of IVD mechanical properties in the progression of curve deformities, however, is still unknown. This is in part due to the difficulties of

measuring IVD properties *in vivo*. Spinal flexibility and intervertebral stiffnesses have been assessed by traction, bending or fulcrum tests [10-12] tests; these techniques, however, do not allow direct measurement of IVD biomechanical properties and require invasive radiographic techniques. Non-invasive techniques based magnetic resonance elastography are being developed to investigate disc mechanical properties [13-15].

A non-invasive and clinical-compatible means of measuring IVD properties could be a useful tool in several orthopedic applications; it could potentially help surgeons and clinicians in determining spine instrumentation levels, detect progressive and stable scoliosis, follow-up physical therapy, etc.

Recently, ultrasound shear wave elastography has been applied to measure cervical IVD *in vivo* [16]. This non-invasive technique [17] allows the measurement of shear wave speed (SWS) in the tissue, which is directly related to the tissue mechanical properties [18]. In particular, a correlation has been reported between SWS *in vitro* measurements and the stiffness and apparent elastic modulus of oxtail functional units (vertebra-disc-vertebra complex) [19].

Lumbar discs, however, are deeper than cervical discs and they are placed beside large arteries (the right and left iliac arteries) pulsating right against it. The aim of the present work was to determine the feasibility and reliability of shear wave elastography to study lumbar intervertebral disc in children and adolescents.

## Methods

### Subjects

Thirty-one healthy children participated in this study ( $11 \pm 3$  years old, ranging from 6 to 17, 16 girls and 15 boys); one girl was excluded because she

had history of surgery of the sigmoid colon, which induced severe intestinal gas accumulation. Both parents signed an informed consent, as approved by the ethical committee (CPP 6001 Ile de France VI).

### IVD Imaging

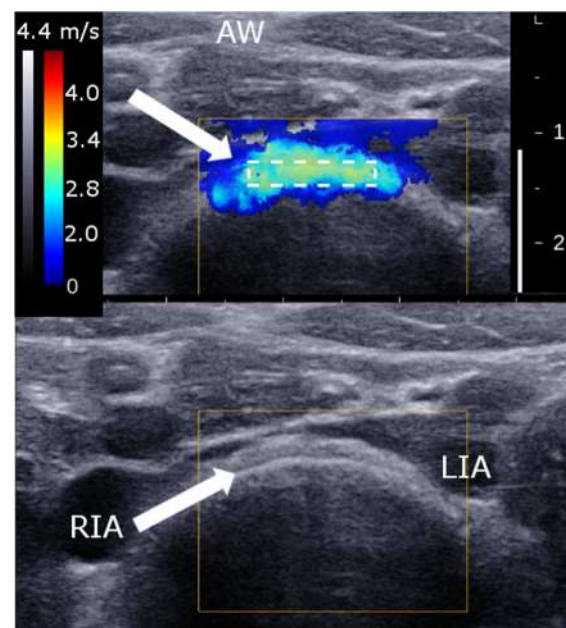
SWS measurements were performed with an Aixplorer (SuperSonic Imagine, France) and either a SuperLinear SL 15-4 ultrasound probe or, if a stable elastographic signal could not be obtained, with a SuperLinear SL 10-2 probe. Imaging depth, focus position and size of elastographic window were adapted for each measurement, while all measurements were performed in “penetration mode”.

The subjects lied supine during the measurement; no particular direction was given about breathing or talking. The probe was placed at the L5-S1 or L4-L5 vertebral level, in the same plane of the disc; this level was determined by observing the distance between the right and left iliac arteries and, if necessary, by following them down from the aortic bifurcation [20]. The vertebral bodies were identified as bright thin round lines around a hypoechoic middle (since ultrasound do not penetrate bone) while the IVD allow some ultrasound penetration (~5 mm, Fig. 1) and therefore is more echogenic. In several cases, *lamellae* were clearly visible (Fig. 1). Once the IVD was found, the probe was rotated and tilted to look for the IVD middle plane, where the disc was more echogenic and the elastographic signal was relatively smooth and stable.

### Protocol

Three clips of about 10 seconds were recorded for each subject (~10 elastographic frames per clip). Data was post-processed using custom software to allow selection and semi-automatic tracking of a region of interest (ROI), which was placed in the *annulus fibrosus* (Fig. 1) in each elastographic frame, as

previously described [19]. Images with too much noise or saturation in the elastographic chart were discarded. The space-average SWS was calculated in the ROI of each frame, then the time-average SWS was calculated in all frames of a given clip, and then the subject average was calculated over the three clips. Time of measurement was retrieved from images time stamp.



**Fig. 1** Example of elastographic image (top frame) and ultrasonography (bottom frame) of lumbar intervertebral disc in an 8 years old girl. The *annulus fibrosus* is indicated by the white arrow (*lamellae* are visible). Abdominal wall (AW) is visible in the top of the image. Right and left common iliac arteries (RIA and LIA) are also visible. The dashed rectangle is the region of interest (ROI) in the *annulus fibrosus*.

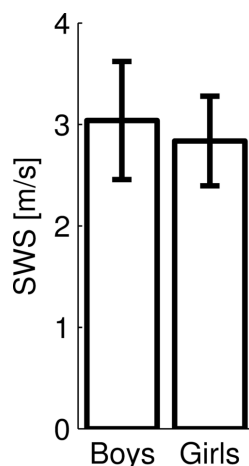
### Reliability and statistics

Measurement reliability (intra-observer repeatability and inter-observer reproducibility) was assessed on a subset of 5 children (average age:  $10 \pm 2$  years). Six measurements (i.e. six 10-seconds clips) were repeated by three experienced operators in random order. The probe was repositioned after each measurement. Repeatability and reproducibility were determined according to ISO 5725 standard. Intraclass correlation coefficient

(ICC) was also determined both intra- and inter-operator; an ICC greater than 0.75 was considered to signify good agreement. Probe effect was determined by repeating measurements with both a high (SL 15-4) and low frequency (SL 10-2) probe on five children. Differences were analyzed with Wilcoxon rank sum tests and correlations were quantified with Spearman's rank correlation coefficient; significance was set at 0.05.

## Results

Intra-operator repeatability was 0.22 m/s (7.5 %) while inter-operator reproducibility was 0.25 m/s (8.7 %) for a single measurement. Repeating three measurements and averaging the result allowed lowering repeatability and reproducibility confidence intervals to 5.0 % and 6.2 % respectively. Intra-operator ICC was higher than 0.9 for each operator (0.98, 0.98, and 0.96, respectively), while inter-operator ICC was 0.98. Difference between measurements performed with the two probes (Table 1) was lower than percentage measurement uncertainty.



**Fig. 2** Shear wave speed (SWS) in boys (n = 15) and girls (n=16).

Average SWS in lumbar IVD was  $2.9 \pm 0.5$  m/s, ranging from 1.8 to 3.9 m/s. SWS difference between girls ( $2.8 \pm 0.5$

m/s) and boys ( $3.0 \pm 0.6$  m/s) was not significant (Fig. 2,  $p = 0.19$ ).

No significant correlations were observed between SWS and subjects' age (Fig. 3a), weight (Fig. 3b), or height (Fig. 3c) or time of measurement.

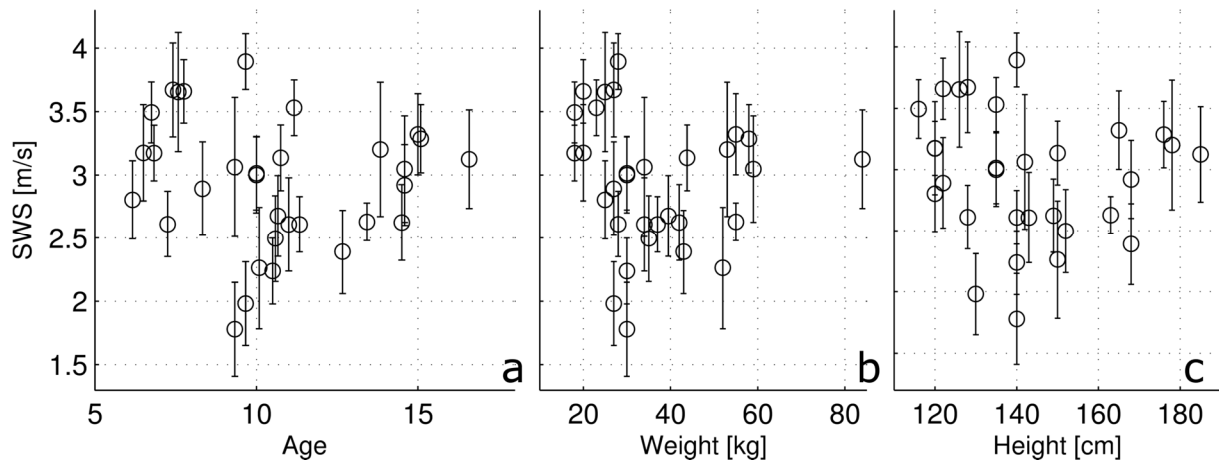
## Discussion

Biomechanical characterization of the intervertebral disc (IVD) *in vivo* could represent a novel non-invasive biomarker [13] for a range of spinal pathologies. Investigations in this sense, especially in the younger population, have been slowed down by the difficulties of assessing disc mechanical properties *in vivo*. In research, *in vitro* testing are informative on IVD properties, but cadaveric studies are usually performed in specimens harvested from older population. Moreover, *in vitro* testing presents limitations such as specimen preparation, hydration, conditioning and preload, etc. [21-23].

In particular, it is well known that IVD properties depend on loading history [24], to the extent that significant changes in stature resulting from disc shrinkage and recovery, can be measured, especially in the lumbar region, over a 24-hour period or following short-term spinal loading [25; 26]. This aspect was not accounted for in the present study, since the time of measurement was recorded but not controlled, as well as the activity of the child before the session. However, no major effect due to session time of day was observed. Circadian variations of disc mechanical properties could be more thoroughly investigated with this technique, together with the effects of physical activity or posture.

The mechanical information conveyed by IVD elastography needs further investigation. From a theoretical point of view, the SWS measured in the *annulus* is directly related to the shear modulus in the plane of the image (i.e., the transverse plane of the disc), with a





**Fig. 3** Relationship between shear wave speed (SWS) and subjects' age (a), weight (b) and height (c). Bars represent subject standard deviation.

polarization parallel to the *lamellae* [18]. In other words, SWS measurements should be directly related to the tangent shear modulus of the annulus,  $\mu_T$ , by the following equation:  $\mu_T = \rho \cdot SWS^2$ , where  $\rho$  is the tissue mass density (usually considered equal to 1000 kg/m<sup>3</sup>). No simple theoretical relation exists between this particular mechanical parameter and the global behavior of the functional unit. However, an *in vitro* study on oxtail samples showed correlations between SWS measurement in IVD transverse plane and the functional unit compressional behavior [19].

Measurement reliability and inter-observer agreement for a single measurement was similar to the one previously obtained in adult cervical disc (0.2 - 0.3 m/s) and in *in vitro* measurements (0.2 - 0.4 m/s) [16; 19], and it was similar or better than those obtained in elastographic evaluation of other soft tissues (for instance, 6.8 % in lower limb muscles [27], 15.6 % in tendon [28], ICC = 0.87 in breast masses [29]). No operator effect was observed, which is not surprising since, unlike muscles, IVD is a deep structure and its measurement is not affected by probe pressure.

The measurement session for one IVD lasts about 5-10 minutes; first it is necessary to find the anatomical landmarks

(i.e. the bifurcation of the aorta to be followed down the right and left iliac arteries, Fig. 1). Then, the position of the intervertebral disc must be determined, and its orientation, in order to obtain a reliable signal (i.e., stable). This last part can last a few minutes because the intestinal contents, gas in particular, can strongly attenuate and reflect ultrasound waves. The continuous pressure of the probe, however, helps displacing these contents while at the same time allowing the probe to get nearer to the disc. One girl was excluded from the study because a stable elastographic image of her L5-S1 disc could not be obtained. This girl had large quantities of intestinal gas, producing well-known “comet tail” artifacts [30], which could not be displaced even after a few minutes of probe pressure.

One measurement (a 10 second clip) takes a few seconds; therefore, it is advisable to adopt as standard protocol to repeat at least 3 measurements in order to average the results and thus significantly reduce the measurement confidence interval.

Measurements were initially performed in all patients with a high frequency probe (SL 15-4, 4-15 MHz bandwidth); if a stable elastographic measure in the disc was not obtained, the lower frequency probe (SL 10-2, 2-10 MHz bandwidth) was used. The former

**Table 1.** Shear wave speed measurements in lumbar annulus performed with a high (SL 15-4) and low (SL 10-2) frequency probe in five children. Differences were lower than percentage uncertainty of measurement.

| Subject          | #1            | #2            | #3            | #4            | #5            |
|------------------|---------------|---------------|---------------|---------------|---------------|
| SL 15-4 (m/s)    | $3.9 \pm 0.2$ | $2.1 \pm 0.1$ | $3.7 \pm 0.3$ | $3.2 \pm 0.2$ | $2.6 \pm 0.2$ |
| SL 10-2 (m/s)    | $3.6 \pm 0.1$ | $2.2 \pm 0.3$ | $3.5 \pm 0.2$ | $3.4 \pm 0.2$ | $2.8 \pm 0.3$ |
| Difference (m/s) | 0.3 [6.8 %]   | 0.1 [7.1 %]   | 0.1 [3.7 %]   | 0.2 [7.2 %]   | 0.2 [8.4 %]   |

yields higher resolution images, but the high frequency ultrasound waves it generates are more attenuated and in some cases shear waves were not properly generated in the disc (i.e., the elastographic signal was absent). In those cases, the lower frequency probe was employed, since the ultrasound waves it generates penetrated more deeply in the tissue, albeit yielding lower resolution images. Results showed that for quantitative results the two probes can be used alternatively since they yield similar SWS values (Table 1).

Previous measurement in adult cervical disc showed an average SWS of  $3.0 \pm 0.4$  m/s; SWS was higher for the younger adult population ( $3.3 \pm 0.3$  m/s for 22-30 years old) than the older ( $2.7 \pm 0.3$  m/s for over 50 years old) [16]. Lumbar disc in children yielded similar average value in the present study ( $2.9 \pm 0.5$  m/s); although the inter-subject variability indicated by the standard deviation is higher in children lumbar IVD (0.5 m/s against 0.3 m/s in adult cervical IVD), the extreme values were similar in the two populations (1.8 and 3.9 m/s in the present study against 2.2 and 3.9 m/s in adult cervical IVD).

The results of this work are promising, and they open the way for a new non-invasive mean of investigation for lumbar disc that shows the potential of becoming a biomarker for spine pathologies in children.

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## Conflict of interest statement

Jean-Luc Gennisson is a scientific consultant for SuperSonic Imagine, and Mickael Tanter is cofounder and shareholder of SuperSonic Imagine (Aix-en-Provence, France). The other authors do not have any conflicting financial interests.



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