Purpose: To examine whether x-ray vector radiographic (XVR) parameters could predict the biomechanically determined vertebral failure load.

Materials and Methods: Local institutional review boards approved the study and donors provided written informed consent before death. Twelve thoracic vertebral bodies were removed from three human cadavers and embedded in resin. XVR measurements were performed by using a Talbot-Lau grating interferometer with the beam direction in anterior-posterior and lateral direction. The mean anisotropy and the mean local average scattering power were calculated for a region of interest within each vertebra. Trabecular bone mineral density (BMD) was determined in each vertebra by using a clinical multidetector computed tomographic scanner. Failure load of the vertebral bodies was determined from destructive biomechanical tests. Statistical analyses were performed with statistical software with a two-sided P value of .05 to calculate Pearson correlation coefficients and multiple regression model.

Results: Statistically significant correlations (P < .05) for failure load with XVR parameters in the lateral direction (r = −0.84 and 0.68 for anisotropy and local average scattering power, respectively) and for failure load and anisotropy in anteroposterior direction (r = −0.63) were found. A multiple regression model showed that the combination of the local average scattering power in lateral direction and BMD predicted failure load significantly better than BMD alone (adjusted R = 0.88 compared with 0.78, respectively; P < .001).

Conclusion: The study results imply that XVR can improve the prediction of osteoporosis.
Osteoporosis is recognized by the World Health Organization as one of the most challenging diseases to the public health systems (1). Approximately 75000000 people are affected by osteoporosis in Europe, North America, and Japan, and this number is expected to increase three-fold within the next 50 years (2). The disease is characterized by a decrease in bone mass and by micro-architectural deterioration, which causes an increase in fracture risk (1).

The current diagnosis method uses dual x-ray absorptiometry to determine the bone mineral density (BMD) and T score (1–3). However, this technique only accesses the bone mass, and several studies have found an overlap in T score and BMD values of patients with and without osteoporotic fractures (4,5).

Bone strength reflects the integration of BMD and bone quality, and BMD accounts for approximately 70% of bone strength (6,7).

The method lacks the capability to assess the microstructure of the trabeculae, which is crucial for bone quality. The trabecular structure of osteoporotic bone shows a dominant alignment in the longitudinal direction and a lack of trabeculae in the transverse direction because the structure compensates for bone loss; it adapts to daily required loads and therefore becomes an anisotropic structure, which means that it is more vulnerable to unexpected loads (8–10).

We intended to analyze this increase in anisotropy and decrease in bone mass with a recently developed technique called x-ray vector radiography (XVR), which is also known as directional dark-field imaging (11,12). XVR developed from grating-based x-ray imaging (13–15), an imaging method that provides complementary information compared with conventional absorption imaging through differential phase-contrast and dark-field imaging. The latter relies on scattering from structures on the submicrometer- or micrometer-length scale. Most importantly, it provides information on structures that are smaller than the actual pixel size (15). XVR uses the directional dependency of the dark-field signal with respect to the orientation of the gratings in the grating interferometer (11).

By yielding the local average scattering power and the degree of anisotropy of the trabecular structure, XVR is capable of providing an estimate for the relative amount of trabecular structures and of the potential bone strength, even if the trabeculae themselves cannot be resolved by the imaging system (12). This is of great importance because it makes XVR dose-compatible with in vivo measurements. Furthermore, it was confirmed (16) that the scattering signal stems from the edges of the trabeculae and therefore gives information on the bone microstructure.

Our study tested whether a correlation between XVR-based parameters and biomechanical properties exists for intact human vertebral bodies. The purpose of our study was to examine whether XVR parameters could predict the biomechanically determined vertebral failure load.

**Materials and Methods**

**Approval by Institutional Review Boards**
Our study was reviewed and approved by the local institutional review boards. The donors had dedicated their bodies for educational and research purposes, and provided written informed consent prior to death, in compliance with local institutional and legislative requirements.

**Sample Acquisition and Preparation**
Twelve intact vertebrae from the thoracic spine (between T6 and T11) were obtained from three human cadavers (four vertebrae from each donor). Two of the donors were men (aged 46 and 62 years at death) and one was a woman (aged 74 years at death). All of the donors had normal body mass index. Donors with a history of pathologic bone changes other than osteoporosis (ie, bone metastases or metabolic bone disorders) were excluded at the outset.

The vertebrae were separated and the intervertebral discs, the posterior ends, surrounding muscle and fat tissue were removed. Each of the vertebral bodies was then embedded in resin (Rencast Isocyanat and Polyol;...
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Figure 1

(a) The vertebrae were scanned in a clinical multidetector CT scanner together with a calibration phantom to determine the trabecular BMD. (b) The Talbot-Lau grating interferometer was used for the XVR scans. It consists of an x-ray source, three gratings (G0, G1, and G2) separated by distances (d and L), the sample (S) in the Eulerian cradle, and the detector (D). The sample is rotated around the optical axis (red arrow) and, upon rotation, the visibility curve oscillates as a function of \( \omega - \phi \), which is the angle between the orientation of the structure (\( \phi \)) and the orientation of the grating lines (\( \omega \)). (c) Image shows the experimental setup for the XVR scans. Shown are (from front to back) grating G1, the sample mounted in the Eulerian cradle, grating G2, and the detector. (d) Image shows a sample of a destructive biomechanical test that was performed to determine the failure load of the vertebral bodies.

Huntsman Group, Bad Säckingen, Germany) up to 2 mm below and above their vertebral endplates. The embedding was performed with parallel alignment of the endplates of the vertebral body with the outer surface of the resin chock to ensure strict axial loading condition of the vertebra during the uniaxial biomechanical tests.

**Determination of BMD**

The BMD of the vertebral bodies was determined from a clinical multidetector computed tomographic (CT) scan by using a whole-body 256-row CT scanner (iCT; Philips Medical Care, Best, the Netherlands); the vertebral bodies were degassed in a sodium chloride solution for at least 3 hours before the CT scan to prevent air artifacts. We applied a tube voltage of 120 kVp with a tube load of 585 mAs. The image matrix was \( 1024 \times 1024 \) pixels, with a field of view of 150 mm that covered the entire vertebra and phantom (Mindways Phantom; Mindways Software, San Francisco, Calif) on the table mat, which yielded an...
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Figure 2

(a) XVR image of one of the samples. To represent both the orientation and the anisotropy of the structure, the color wheel is used for encoding, and orientation is encoded as color (e.g., vertical structure corresponds to red color) and anisotropy is encoded with the brightness (black corresponds to no anisotropy, brightest or strongest color to highest anisotropy). The upper white rectangle shows how the regions of interest were chosen for the samples. (b) Multiple XVR images of three different samples with the color coding shown in a. The first column of images displays the anteroposterior scans, while the second column displays the lateral scans. The samples are ordered by failure load, determined from the destructive tests that increased in failure load from top to bottom. Displayed are samples B1, C1, A1 (from top to bottom, respectively).

interpolated voxel size of $146 \times 146 \times 300 \, \mu m^3 = 6.4 \times 10^6 \, \mu m^3$. A dedicated calibration phantom (Mindways Phantom; Mindways Software) was scanned together with the vertebral bodies (Fig 1a). The trabecular BMD was calculated by a radiologist who used the calibration phantom (Mindways Phantom; Mindways Software) with software developed in-house based on data language (Interactive Data Language; Research Systems, Boulder, Colo) to convert the pixel attenuations, given in Hounsfield units, into bone mineral calcium hydroxyapatite, which was outlined in a previous study (17).

**XVR Technique**

The technique of XVR employs the imaging modality of the dark-field signal (14) obtained from x-ray grating interferometry (13,18,19). A detailed description of x-ray grating interferometry can be found in the Appendix (online). For XVR (11,12), the sample (or, alternatively, the gratings) is rotated around the optical axis in a standard Talbot-Lau grating interferometer setup (Fig 1b, 1c). The scattering is described by the visibility (14). As the grating interferometer is only sensitive to scattering perpendicular to the grating lines, the visibility curve oscillates on the rotation. The negative logarithm of the resulting visibility curve ($V$) can be approximated by the following Fourier expansion (20):

$$-\ln[V(\omega)] = b_0 + b_1 \cos[2(\omega - \phi)],$$

where ln represents the logarithm, $V$ represents the visibility, $\omega$ represents the orientation of the grating lines, $b_0$ and $b_1$ are the first two Fourier components, cos represents cosine, and $\phi$ represents the structure. The average local scattering power (also called average dark-field signal) and the anisotropy (or degree of orientation) are defined by the first two Fourier components as $b_0$ and $b_1/b_2$, respectively. The difference of $\omega - \phi$ equals the angle between the orientation of the structure ($\phi$) and the orientation of the grating lines ($\omega$); in other words, $\phi$ is the preferred structural orientation relative to the direction of the grating lines.

**XVR Images**

The images were obtained by using a Talbot-Lau grating interferometer that consisted of three gratings. A schematic drawing of the setup is in Figure 1b, while Figure 1c shows a photograph of the apparatus. Two of the gratings were absorption gratings (G0 and G2) with a silicon substrate height of 500 $\mu m$ and 150 $\mu m$, respectively, and gold lines that were 160–170 $\mu m$ in height filled with photoresist (SU-8). The phase grating (G1) was fabricated...
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Figure 3

**Figure 3:** XVR images with color coding. To represent both the orientation and the anisotropy of the structure, the color wheel is used for encoding, and orientation is encoded as color (e.g., vertical structure corresponds to red color) and anisotropy is encoded with the brightness (black corresponds to no anisotropy, brightest or strongest color to highest anisotropy). The anteroposterior (AP) scans are displayed in the top three rows and the lateral (L) scans in the three bottom rows. Each row displays the four vertebrae from each donor. The donors are indicated by the letters A, B, and C, and the four vertebrae from each donor are indicated by numbers 1–4.

The x-ray source was a high-power x-ray tube (MXR-160HP/11; Comet AG, Flamatt, Switzerland), and it was operated at 60 kVp and 30 mA with an exposure time of 1 second. A 3-mm aluminum filter was used. The sample was placed 40 cm downstream of G1 in a cradle.
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Table 1

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Adjusted P Value</th>
<th>Pearson Correlation Coefficient</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Correlations with failure load</td>
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<td></td>
<td></td>
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<tr>
<td>BMD</td>
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<tr>
<td>Average anisotropy AP</td>
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<td>.021</td>
</tr>
<tr>
<td>Average local scattering power L</td>
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<td>0.677</td>
<td>.016</td>
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<tr>
<td>Average anisotropy L</td>
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<td>Multiple regression model</td>
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<tr>
<td>BMD alone</td>
<td>−0.780</td>
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<td>&lt;.001</td>
</tr>
<tr>
<td>BMD and average local scattering power L</td>
<td>−0.876</td>
<td>NA</td>
<td>&lt;.001 .016*</td>
</tr>
</tbody>
</table>

Note.—AP = anteroposterior, L = lateral, NA = not applicable.
* Incremental gain from adding average local scattering power lateral to the model. .016 is the P value for the model with BMD and the best XVR parameter. P value of less than .001 gives the incremental gain from adding the XVR parameter to the model.

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(Enderian Cradle; Huber Diffractionstechnik GmbH, Rimsting, Germany). We used a detector (PaxScan 2520D; Varian Medical Systems, Salt Lake City, Utah) with a cesium iodide scintillator. The detector has a 127-μm pixel pitch.

For each vertebral body, two XVR images were acquired, one with the beam anteroposterior and the other lateral.

Calculation of XVR Parameters

For the anteroposterior and lateral images, two parameters \( b_0 \) and \( b_0/b_0 \) were calculated as the average over a rectangular region of interest, which was chosen as the largest rectangle that fit between the two resin plates and excluded the strongly scattered region from the edges of the vertebral body. This is illustrated in Figure 2a. The two parameters were the mean local average scattering power \( b_0 \) (or average dark-field signal) and the anisotropy \( b_0/b_0 \).

Destructive Tests

After these radiographic measurements, destructive tests of the vertebrae were performed by using a mechanical testing system (Wolpert Werkstoffprüfmaschinen AG, Schaffhausen, Switzerland), to determine their failure load, illustrated in Figure 1c. After we performed 10 preconditioning cycles with an uniaxial load between 10 N and 400 N at a rate of 5 mm per minute, the failure load was determined by applying uniaxial monotonic compression at the same rate, and vertebral failure load was defined as the first peak of the stress-displacement curve with a subsequent drop of more than 10% (17,21).

Results

XVR Imaging Results

Imaging results from XVR are displayed in Figure 2. The XVR images show a combined representation of the anisotropy and the local structural orientation. This representation is displayed via the color wheel in Figure 2a, where the color indicates the orientation of the structure (eg, blue corresponds to horizontal structure, red corresponds to vertical structure) and the brightness indicates the anisotropy (where brightest color corresponds to the highest anisotropy; toward the outside of the color wheel, the colors get brighter, and the brighter or stronger a color in a certain pixel is, the anisotropy is higher; the center is black, which means no anisotropy).

Figure 2a illustrates how the region of interest for the analysis of dark-field parameters was chosen. Figure 2a displays the XVR image of the whole vertebra with its embedding of the vertebral body in resin, and the rectangle highlights the region that was selected for analysis (also shown magnified).

Figure 2b displays the chosen regions of interest for three vertebra (one exemplary sample from each donor) imaged for anterior-posterior (left side of the image) and lateral (right side of the image) orientation each. The color coding is according to the color wheel in Figure 2a and is described. It is visible from the images that the orientation of the trabeculae within the vertebral body is predominantly in the vertical direction (ie, red color). The images are ordered according to ascending failure load from first to third row. Additionally, XVR images of all vertebral bodies are provided in Figure 3.

Statistical Analysis

Statistical analyses were performed with statistical software (SPSS; SPSS, Chicago, III) and were graphically evaluated (Origin; OriginLab, Northhampton, Mass). All tests were performed by using a two-sided P value of .05. The Kolmogorov-Smirnov test showed no significant differences from a normal distribution for all parameters. Correlations between two parameters were evaluated with the Pearson correlation coefficient (r). Multiple linear regression analysis was performed to assess whether the combination of XVR parameters and BMD could predict failure load significantly better than BMD alone. XVR parameters were included in the regression model in a forward procedure if the P value was less than .05. Adjusted regression coefficients were calculated for each model. Models were compared by using the extra sum-of-squares F test.
local scattering power and average anisotropy for anteroposterior and lateral orientations each) with the failure load. The correlation of BMD with failure load was statistically significant ($P < .001$).

For the XVR parameters, we found one statistically significant correlation for the average anisotropy (lateral; $P = .001$). The correlations of average anisotropy (anteroposterior) and average local scattering power (lateral) also were found to be statistically significant ($P < .05$). A trend was determined for the average local scattering power (anteroposterior; $P = .08$).

These correlations are illustrated in plots of failure load against the four calculated XVR parameters, which are shown in Figure 4. As highlighted by the plots, failure load was found to increase with increasing average local scattering power and to decrease with increasing anisotropy. Additionally, all results from BMD measurements, destructive tests, and XVR are provided in Table 2.

The best multiple regression model was found for the combination of BMD and the average local scattering power (lateral). The combination of these two parameters predicted failure load significantly better than BMD alone (adjusted $R = 0.88$ vs adjusted $R = 0.78$; $P < .001$). All other XVR parameters were not included in the model, since their corresponding $P$ values did not reach statistical significance. Note that adjusted $R$ value is smaller than the actual $R$ value because it adjusts for

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**Figure 4**: Four plots show statistically significant linear correlations between parameters obtained from XVR and failure load. The lines show the linear fits to the data points in each plot. Shown are plots of failure load against the average local scattering power in (a) anteroposterior (AP) direction and (b) lateral (L) direction, and the average anisotropy in (c) anteroposterior and (d) lateral direction. Positive correlations were found for the local average scattering power (average dark-field signal) and negative correlations were found for anisotropy.

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  - **Figure 4**: Four plots show statistically significant linear correlations between parameters obtained from XVR and failure load. The lines show the linear fits to the data points in each plot. Shown are plots of failure load against the average local scattering power in (a) anteroposterior (AP) direction and (b) lateral (L) direction, and the average anisotropy in (c) anteroposterior and (d) lateral direction. Positive correlations were found for the local average scattering power (average dark-field signal) and negative correlations were found for anisotropy.
added variables to the model and increases only if the new variable more than coincidentally improves \( R \) values.

### Discussion

We examined the relation between failure load and two average XVR parameters (anisotropy and local average scattering power) for 12 human vertebrae. In summary, the analysis of the experimental results shows that failure load correlates strongly with laterally measured parameters, and the mean local average scattering power and the mean anisotropy. The anteroposterior plots show similar characteristics in principle, but the correlation coefficients are lower and the correlation for the average local scattering power is not statistically significant (\( P > .05 \)).

Failure load shows a positive (ie, increasing) relationship with the local average scattering power. By using the local average scattering power as an indicator for the total number of structures present, it appears reasonable that higher local average scattering power (ie, a higher number of structures) would increase the stability of the structure (ie, higher failure load).

A negative (ie, decreasing) linear correlation of failure load with the anisotropy was found, with a strong correlation for the lateral direction. These results are plausible as bone develops a more anisotropic structure when aging and becomes weaker, which was outlined in the introduction. Visual inspection also shows the preferable alignment of the trabeculae in the longitudinal direction.

Furthermore, the fact that the local average scattering power (lateral view) improved the correlation of failure load with BMD in a multiple regression model contributes to the assumption that XVR does provide complementary information on bone stability compared with BMD.

An important aspect is that the presented technique could, in principle, be dose-compatible with clinical applications. The pixel size used for the results presented in our study was 127 \( \mu \text{m} \), which is in the range of clinical feasibility, and the resolution could probably be further decreased to facilitate clinical application. Pottdevin et al (12) previously showed that pixel sizes up to 500 \( \mu \text{m} \) could still provide information on anisotropy and preferred structural orientation. The influence of the pixel size has not yet been examined with respect to the quantitative analysis presented in this study and how far the number of steps and angles for the XVR scan could be reduced, and these would need to be assessed in another study.

Our study was limited to a moderate number of samples (\( n = 12 \)). It appears promising to conduct a further study with a higher number of vertebrae to obtain more diagnostically conclusive results. Furthermore, the study has been performed on isolated vertebrae only. First, this ignores the influence of the intervertebral discs on mechanical properties, such as failure load. Therefore, a next step might be to test the hypothesis on a segment of vertebrae. Second, the influence of the surrounding body tissue on XVR measurements (because XVR is a projectional technique) has not been investigated yet. These are steps that need to be considered for clinical applications, and dose constraints should be examined in further studies.

To conclude, in our study, failure load correlated with the average anisotropy and the average local average scattering power, and a combination of XVR and BMD provided a better prediction of failure load compared with BMD alone. These findings support the conclusion that XVR may have future effect on the prediction of bone strength, especially with respect to the diagnosis of osteoporosis. XVR can significantly
improve the established method as it applies to BMD for the prediction of bone strength as in the diagnosis of osteoporosis.

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