Valgus deformity induced by asymmetrical compression of the growth plate in lambs

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SUMMARY
An asymmetrical load exerted on the growth plate induces a deformity (valgus or varus). The respective roles of amount of force and duration of compression in the mechanism of deformity have not been completely elucidated.

Our experiments were carried out to (i) induce an experimental valgus deformity in lambs, (ii) asymmetrically delay the growth without stopping it or injuring the growth plate, (iii) identify the role of compression duration on valgus deformity.

A total of 12 eight-week-old lambs were used. A controlled asymmetrical compression force was applied on the distal growth plate of the radius by two Kirschner wires connected by springs located medially and laterally. The loading period lasted 9 weeks. Length of the radius, distances between pins and angles between articular surfaces of the radius were measured and compared to controlateral radius. A quantitative histological study was performed.

At the end of the loading period, a weak difference in length was observed between the loaded radius and the control radius (< 1.5 % of radius length). Angle between articular surfaces increased in loaded limbs whereas angle of control limbs remained constant (7° of deformity after six weeks of compression). No histological lesion were observed.

A valgus deformity was performed by delayed growth without injury to the growth plate. This model affords good opportunities for studying the mechanical factors and mechanism which result in valgus deformity and its development.

KEY-WORDS : growth plate - asymmetrical pressure - delayed growth - valgus deformity.

RÉSUMÉ
Élaboration d’une déformation en valgus par compression asymétrique du cartilage de croissance chez l’agneau. Par P. COLLARD-MEYNAUD, D. MATHON, E. ASIMUS, R. DARMANA, P. FRAYSSINET, J.-Ph. CAHUZAC et A. AUTEFAGE.

Une compression asymétrique, appliquée sur un cartilage de croissance, induit une déformation angulaire (en valgus ou varus). Les rôles respectifs de l’intensité de la force et de la durée de compression dans les mécanismes de développement d’une déformation restent encore mal éclairés.

Nos expérimentations ont été menées dans le but d’induire expérimenteralement une déformation angulaire en valgus chez l’agneau, par ralentissement asymétrique de la croissance et sans induire les lésions irréversibles au sein du cartilage de croissance, et enfin, d’identifier les effets de la durée de compression sur le développement de la déformation.

Une force de compression asymétrique a été appliquée sur le cartilage de croissance distal du radius chez 12 agneaux, âgés de 8 semaines. La force a été exercée par deux broches de Kirschner implantées de part et d’autre du cartilage de croissance et reliées entre elles par deux ressorts médial et latéral. L’étude a duré 9 semaines. La longueur du radius, les distances entre les broches et les angles entre les broches et les surfaces articulaires du radius ont été mesurés et comparés au membre contralatéral. Une analyse histologique quantitative a été réalisée.

Au terme de la compression, l’angulation entre les surfaces articulaires a augmenté sur le membre comprimé alors qu’il est resté constant sur le membre témoins (7° de déformation après 6 semaines de compression). Le mécanisme à l’origine de cette déformation a été une croissance asymétrique induite par un ralentissement de l’activité du cartilage de croissance en région latérale par rapport à la région médiale. Aucune lésion histologique n’a été observée.

Ainsi, une déformation en valgus a été induite par ralentissement asymétrique de la croissance. Le modèle développé permettra, à l’avenir, d’étudier les différents facteurs mécaniques ainsi que les mécanismes à l’origine de la mise en place et du devenir spontané d’une déformation angulaire.

MOTS-CLÉS : cartilage de croissance - compression asymétrique - déformation en valgus.
Introduction

In 1829, Delpech was the first author to describe the effect of mechanical pressure on growth plate behaviour from clinical studies: increased pressure on the physeis is able to inhibit growth, whereas decreased pressure increases it.

Experimental studies of symmetrical compression of the growth plate have shown that the main factors influencing growth activity are the amount of force and duration of compression.

Major pressure can stop growth in length [30], whereas minor pressure has no influence on growth [7, 8, 30], and moderate pressure can delay growth without stopping it [4-7, 11, 13, 14, 28, 29]. This decreased growth is the outcome of delayed activity of the loaded physeis as a whole [4, 12, 29]. Authors investigating the duration of compression noted a threshold (3 weeks in rabbits) above which there was premature closure of the growth plate [5, 6, 11, 13, 14, 28, 29].

When an asymmetrical load is exerted on growth plate, it induces a deformity (valgus or varus) due to asymmetrical growth of the physeis [4-6, 22, 28]. The respective roles of amount of forces and duration of compression in the deformity mechanism have not been completely elucidated.

The purposes of our experiments were to:
(i) induce an experimental valgus deformity;
(ii) asymmetrically delay growth without stopping it or injuring the growth plate;
(iii) identify the role of compression duration on valgus deformity.

Materials and methods

A valgus deformity was induced by applying controlled asymmetrical compression forces to the distal growth plate of the radius in lambs. The compression was produced by two Kirschner wires placed above and below the physeis and

![Figure 1](image-url) - Radiographs of loaded (A) and control (B) radii of a lamb. "L" and "M" are respectively lateral and medial sides. On these radiographs, are represented the length of the radius (1), distances between pins on the medial (2) and lateral (3) sides, angle between the two wires (4) and angle between the two joints (5).
connected by springs located laterally and medially [7] (figure 1).

ANIMALS

Twelve eighth-week-old crossed lambs were used. Previous experiments showed that the growth rate of the radius was rapid and linear between eighth and fourteen weeks old [7, 9]. The lambs were fed an industrial lamb diet and water ad libitum. They were kept according to the European guidelines for animal care and accommodation.

SURGICAL PROCEDURE

The lambs were prepared according to a previously described technique [7, 9]. Surgery was performed under general anaesthesia. Two 2.5 mm Kirschner wires were inserted fluoroscopically in the distal radius of each forelimb placed on either side of the growth plate and parallel to the physeal plane (figure 1). One of the two forelimbs was randomly chosen to support the compressive device, which consisted of calibrated steel springs (Groupe CGR, Blagnac, France) fixed to both ends of the Kirschner wires. The wires inserted in the second radius were left unconnected and were cut close to the bone. This radius was used to control the growth in length (figure 1).

SPRINGS

According to experiments in which symmetrical compression had been applied [7, 8], the forces exerted by the springs were expected to delay but not to stop growth. These forces induced an asymmetrical pressure on the entire growth plate and prevented distraction on the medial side. At the beginning and end of the experiments, the springs were calibrated. The laterally exerted force was twice the medial force (20.2 ± 1 N vs 10.3 ± 1.3 N).

RADIOGRAPHIC PROCEDURE AND MEASUREMENTS

Dorsopalmar radiographs of both radii were obtained weekly under standardized conditions [7, 9] with the forelimbs in contact with the radiograph cassette (T-MAT G/RA Film Kodak in a Kodak X-Omatic cassette (Lanex Fine Screens) ; Eastman Kodak Company, NY 14650, USA). The distance between the cassette and the X-ray tube was the same throughout the study in order to eliminate any changes in bone magnification. The radiographs were digitized using a scanner (MIRAGE D-16L - UMAX Data Systems Inc. ; Thetascan, Toulouse, France). Measurements, accurate to 0.1 mm, were performed on all digitalized radiographs using an image analysis software for Macintosh (Optilab 2.6™ ; Graftek France, Recherche et Valorisation des Matériaux, Toulouse, France).

1: Resting zone
2: Proliferative zone
3: Hypertrophic zone

**Figure 2.** — Shape of the physis and zones of interest. Two zones of interest were determined: one in the medial zone (a) and one in the lateral zone (b). All histomorphometric measurements were realised in these areas.
Mirmande, France). The following measurements were obtained from each radiograph (figure 1):

- radial length measured parallel to the long axis of the bone between the most proximal edge of bone to the most distal tip of bone;

- distances between the pins on the medial and lateral sides, measured between the points where the pins penetrated the cortical bone;

- angle between the two wires (WA);

- angle between the two joints (JA) : angle between proximal and distal surfaces of the radius. Each line was drawn between the lateral and medial borders of the articular surfaces.

The growth contribution of the distal growth plate was obtained by measuring the distance between the wires on the medial and lateral sides at the time of the operation and subtracting it from the distance measured at the appropriate time thereafter. By comparison of the JA and the WA on the experimental and control radii, it was possible to estimate the extent of angular deformity. All measurements were obtained by the same operator throughout the study.

Half of the lambs were euthanatized with an overdose of barbiturate at the end of the loading period. All radii were excised, dissected clean and prepared for histological study.

**HISTOLOGICAL ANALYSIS**

The radius was sawn at the metaphyseal level. The samples were fixed in a 4 % paraformaldehyde solution buffered in PBS for one week at room temperature. They were dehydrated in increasing ethanol solution. They were, then, embedded in PMMA, and 3 \( \mu \)m thick sections cut using a Reichert type E microtome were performed. The sections were stained with Giemsa associated or not with the Von Kossa method for undemineralized tissue.

The shape of the cartilage was observed, especially the persistence of the different histological structures (resting, proliferating and hypertrophic zones) composing the growth plate.

The physis were cut by half in the latero-medial plane and the histological sections were performed at this level. Two zones of interest were determined from which the histomorphometric measures were taken. They were located 3 mm far from the condylar radius edges (figure 2). The measures were taken at \( x8 \) for the whole cartilage thickness, and \( x20 \) for the thickness of the different zones of the growth plate. The measures of the cell surface were done at a \( x120 \) magnification.

The different parameters concerning the growth plate thickness included:

- the thickness of the total cartilage,
- the thickness of the resting zone,
- the thickness of the proliferative zone,
- the thickness of the hypertrophic zone.

The surface of the different zones of interest was specified manually to the computer then the thickness was obtained by dividing the surface by the length.

The surface of the septa containing the cells and the cell density were obtained by thresholding and calculated auto-

<table>
<thead>
<tr>
<th></th>
<th>Control limb</th>
<th>Loaded limb</th>
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<tbody>
<tr>
<td></td>
<td>Medial</td>
<td>Lateral</td>
</tr>
<tr>
<td>Thickness (( \mu )m)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>618 ± 44</td>
<td>570 ± 48</td>
</tr>
<tr>
<td>Resting zone</td>
<td>115 ± 35</td>
<td>99 ± 22</td>
</tr>
<tr>
<td>Proliferating zone</td>
<td>289 ± 36</td>
<td>269 ± 64</td>
</tr>
<tr>
<td>Hypertrophic zone</td>
<td>214 ± 33</td>
<td>203 ± 24</td>
</tr>
<tr>
<td>Cell density (nb/mm(^2))</td>
<td>1232 ± 208</td>
<td>1210 ± 275</td>
</tr>
<tr>
<td>Cell number/columns</td>
<td>30.8 ± 4.6</td>
<td>25.6 ± 3.8</td>
</tr>
<tr>
<td>Surface (( \mu )m(^2))</td>
<td>327 ± 48</td>
<td>322 ± 84</td>
</tr>
</tbody>
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**Table 1.** — Histological measurements. Thickness of the total growth plate and its different zones : it was obtained by dividing the surface by the length of each zone. Cell density : it was obtained in the resting zone by thresholding and calculated automatically by the computer. Number of cells per columns : the count of the proliferating cells were performed in ten columns. The mean of the ten columns is represented. Surface of the hypertrophic cells : the mean surface of the chondrocytes was measured in the hypertrophic zone.
FIGURE 3. — Temporal evolution of lateral and medial distances. The mean difference between wires radiographic distances (mm) on the lateral and medial sides are plotted against time (weeks), comparing the loaded and control limbs. The mean difference between wires radiographic distances was obtained by measuring the distance between the wires on the medial and lateral sides at the time of the operation and subtracting it from the distance measured at the appropriate time thereafter.

FIGURE 4. — Temporal evolution of angles. The mean difference of angles (JA and WA) are plotted against time (weeks), comparing the loaded and control limbs. The mean difference of angles was obtained by measuring the angles between the joints and between the wires at the time of the operation and subtracting it from the distance measured at the appropriate time thereafter.
matically by the computer. The number of the cells in the columns was the mean of the cells counted in ten columns.

**STATISTICAL ANALYSIS**

The values for the loaded and control forelimbs were compared and plotted. Quantitative data are expressed as mean ± standard deviation.

An ANOVA for repeated measures was used to detect any significant differences between loaded and control values. When significant differences were found, a Student’s t-test was performed. The selected level of significance was 0.05.

A linear regression analysis relating growth variables to time was fitted to the data.

Statistical analysis was performed using a special software for Macintosh (Systat 5.2™; Systat, Evanston, U.S.A.).

**Results**

The lambs did not show any signs of discomfort after the operation (no lameness was observed). All animals developed a clinical valgus deformity.

**LENGTH OF THE RADIUS**

The over-all length of the loaded radii was reduced compared to the control radii (1.5% of radius length) (p < 0.05). The increase in growth was 22.8 ± 3.2 mm in loaded limbs and 25 ± 3 mm in control limbs.

**VALGUS DEFORMITY**

Measurements of distances between pins

At the beginning of the study, the medial wire distances were 23.1 ± 1.3 mm in loaded limbs vs 22.0 ± 2.0 mm in control limbs. The lateral wire distances were 21.5 ± 0.5 mm in loaded limbs vs 21.7 ± 1.3 mm in control limbs.

In control limbs, the increase in distance between pins did not differ between the lateral and medial sides (p < 0.05) (figure 3). In loaded limbs, the lateral distance between the pins increased more slowly than the medial distance (p < 0.05).

On the lateral sides, the gap between pins in loaded limbs was significantly lower than in the control limbs from the first week of compression onwards (p < 0.05) (figure 3). On the medial sides, the distance between pins in the loaded limbs was significantly lower than in the control limbs from seven weeks of compression onwards (p < 0.05).

Measurements of angles (figure 4)

At the beginning of the study, the JA were 15.6 ± 2.3° in loaded limbs vs 14.5 ± 2.4° in control limbs. The WA were 1.3 ± 1.3° in loaded limbs vs 3.1 ± 3.9° in control limbs.

The slope of the curves increased rapidly in the early six weeks and then slowed down.

The increase in the joint angle (JA) was 6.2 ± 3.3° in loaded limbs and -0.5 ± 1.5° in control limbs. The difference was significant from two weeks of compression onwards. The increase in the wire angle (WA) was 7.2 ± 3.6° in loaded limbs and -0.1 ± 1.5° in control limbs. The difference was significant from the first week of compression onwards.

The comparisons of joint angles (JA) and wire angles (WA) for each limb were not significantly different.

**HISTOLOGY** (table I)

All sample sections showed a normal growth plate structure. The overall thickness of the growth plate did not seem to be decreased in the compressed group. The five different zones of the growth cartilage were preserved when the growth plate was compressed and their thickness did not differ statistically from the control ones. Even the columns of the dividing zone seemed to be unaffected by the compression. The cell density in the resting zone and the number of cells per column in the proliferative zone did not differ in any of the samples. The surface of hypertrophic cells section showed no significant difference between the loaded and the control growth plates.

**Discussion**

During our experiments, the lambs have developed a valgus deformity in which the amount of angulation changed during the study. This deformity was induced by an asymmetrical growth retardation. At the end of the loading period, the difference of the radial length between loaded and control radii was weak, showing that the growth process had been slightly disturbed by the compression (<1.5% of radius length).

In animals, spontaneous valgus deformities are rare. They have occurred in lambs, raised in total confinement or due to nutritional deficiency, and in horses due to intrauterine positioning or primary aberrant or imbalanced growth in the epiphysis [10, 32, 33]. Experimental valgus deformity has been produced in lambs [16, 25, 26], goats [20], calves [27, 30], rabbits [5, 6, 13, 15, 28, 34], rats [23, 24] and dogs [19, 22]. The large size of lambs makes them an ideal model for growth studies. Moreover, they are quiet animals so that radiographs and measurements can be performed without general anaesthesia.

Series of experiments have been reported using different bones such as the radius, femur, tibia or caudal vertebrae. In our experiments, we chose the radius because i) the surgical approach to the distal growth plate is easy, and ii) the forelimb is a straight limb (the radius is a vertical bone) so that forces exerted by body weight-bearing are perpendicular to the growth plates [7, 8].

Several models have previously been described to induce a valgus deformity. An osteotomy of the shaft induces an angulation without delay [1, 19], but bone healing may interfere with activity of the growth plate [31]. An asymmetrical distraction of the growth plate by separation of the epiphysis from the metaphysis [25] leads to a valgus deformity, but it...
can induce injuries of the growth plate (fracture and premature closure) [2, 3, 16, 20, 26]. A lower force of asymmetric distraction will induce a deformity without fracture of the physis, but probably not sufficiently to be of clinical significance [27]. Finally, an asymmetrical compression [5, 6, 11, 13, 21-24] results in:

(i) temporary arrest of the growth plate activity by staples, rigid external fixation [11, 13, 21, 22];

(ii) delayed growth plate activity induced either by postural aberration by surgical division of certain muscle groups [5], or by deformation of the tails with a metal wire to form a loop [24], or with an Ilizarov-type apparatus [23], or by means of a plaster cast [6]. Those compressive devices impaired locomotion or imposed the loading of several growth plates.

In our investigation, the compressive device used has already been described by the authors [7, 8] for applying symmetrical forces to the growth plate. We showed that wires did not disturb growth and did not induce local reaction or radiographic abnormalities. The induced deformity was due neither to temporary arrest, destruction or overgrowth of one side of the growth plate. The valgus deformity occurred in the distal part of the bone and the shaft itself did not bend, or fracture. No premature closure of the physis was observed in our study.

Published findings of histology are not uniform. Some authors described only minor changes which are observed at the physi-metaphyseal junction and characterized by the disturbance of the process of endochondral ossification [6, 17, 32]. Others described marked decrease of the epiphyseal plate thickness on the loaded side, deviation and disorganization of cell columns. They also noticed a retardation or an arrest of mineralization process [4, 13, 18, 28]. Bony bridges are an obvious sign of premature closure and growth arrest. In our study, the measured parameters permitted to evaluate the proliferation of the cells in the proliferative zone and the enlargement of the chondrocytes in the hypertrophic zone which are the two main factors for the long bone growth. We did not observed one of the previously described signs. There was histologically no evidence of change, even though a valgus deformity occurred. We can therefore assume that the valgus deformity was induced by an inhibition of the growth plate cellular activity without morphological disturbances.

The deformity therefore resulted solely from a differential rate of growth, produced by the stresses applied to the growth plate, without injury to the growth plate or any tissues. The compressive device allows normal locomotion without lameness. The angulation increased rapidly in the early six weeks and then slowed down. It thus affords good opportunities for studying the mechanical factors and mechanisms which result in valgus deformity and its development. Owing to the lack of morphological lesion, we can assume that the growth will remain after device removal. More research is required to determine the rate of the remain growth.

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