IS BOVINE COCCYGEAL INTERVERTEBRAL DISC A SUITABLE MODEL TO STUDY HUMAN LUMBAR DISC?

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INTRODUCTION
Aging and degeneration affect the turnover of the proteoglycans and collagens that make up most of the extracellular matrix of intervertebral discs (IVDs) [1]. However, the effect of aging and degeneration on the metabolism of lumbar IVDs is less frequently studied because human discs suitable for in vitro synthesis experiments are difficult to obtain. Bovine coccygeal discs are readily available, in contrast to human discs and represent a common source of tissue in the disc field. It was therefore suggested that bovine coccygeal IVDs could be a suitable alternative to human lumbar discs for in vitro studies because the general properties of these bovine discs are similar to those of the human discs [2]. In this study, the biochemical composition (proteoglycan, type II collagen, and denatured type II collagen) and the cell density (DNA) of the IVDs present in young and adult bovine tails are described in order to determine whether this disc source serves as a useful model for the study of the human lumbar discs. The results are analyzed in relation to disc level, age and tissue region.

METHODS
Intervertebral discs from young steers (8-20 months; n=3) and IVDs from adult bovines (2-4 years; n=3) were processed 2-3 hours after slaughter. All IVDs were classified as nondegenerate grade I according to the grading system of Thompson [3]. The IVDs from the bovine tails were dissected from their adjacent vertebral bodies and into the nucleus pulposus (NP) and the annulus fibrosus (AF). The discs were numbered from the largest coccygeal disc obtained intact from the abattoir to the tip of the tail (fixed as CC9). The percent water content and dry tissue weight were determined after drying the tissues at 80°C for 6 days (until constant weight). The NP and AF of each disc were digested with a-chymotrypsin (Sigma) followed by proteinase K, as previously described [4,6]. DNA was measured using the 1,9-dimethylmethylen blue (DMMB) dye binding assay [5]. Type II collagen was determined from the a-chymotrypsin fraction. DNA content was measured in proteinase K digested fractions [6]. Multivariate analyses of variance were performed with results considered statistically significant at p < 0.05.

RESULTS
Water content did not vary between disc levels but was significantly greater in the younger discs than in the older ones (NP: P<0.0001; AF: P<0.03) (Fig 1A and 1B). As expected, more water was also found in the NP than in the AF (P<0.0001). The proteoglycan content was significantly greater in the NP than in the AF (P<0.0001) (Fig 1C and 1D). More proteoglycan was present in the adult AF than in the young AF (P<0.03), while the NP proteoglycan content remained constant, regardless of disc level or age. Significantly more type II collagen was present in the NP than in the AF, regardless of age and disc level (P<0.0001) (Fig 1E and 1F). More type II collagen was present in adult AF compared to the younger tissue, but this was not statistically significant. Moreover, more percent denatured type II collagen was found in the young IVDs than in the adults (P<0.004), particularly in the case of the NP (P<0.0001). Finally, DNA content was significantly greater in the AF than in the NP (P<0.0001). Interestingly, there was more DNA in the adult AFs than in the young AFs (P<0.004).

DISCUSSION
This work describes the value and limitations of using the bovine tail as a model for the human lumbar spine. The trend of higher water content in the NP compared to the AF appears to be universally similar between human lumbar discs and bovine coccygeal IVDs. The decrease in water content with age observed in the bovine IVDs is also present in human lumbar discs. However, there is a slight increase following the initial decrease in the less than 25 years-old human discs [1].

It has been previously observed that proteoglycan and water contents decrease with disc level (from L1-L2 to L5-S1) in human lumbar discs, while total collagen content remains constant. In bovine coccygeal IVDs, differences in proteoglycan and water contents between disc levels were not statistically significant. Type II collagen content also did not vary between disc levels. The difference in proteoglycan content between the NP and the AF of the bovine coccygeal discs are observed only in the younger human lumbar disc population (up to 25 years old), because with aging, proteoglycan concentration decreases in the NP at a much greater rate than in the inner AF, such that no differences in proteoglycan content is present between the older human lumbar NP and AF [1]. Similarly, the greater denatured type II collagen content in the young bovine NP compared to the adult NP is also observed in human lumbar discs but only in the younger discs [1].

In view of these results, it appears that the young and adult bovine coccygeal IVDs are still in the growth phase and therefore more biochemically comparable to the younger human lumbar discs. However, caution must be taken when extrapolating data from bovine coccygeal discs. For example, in humans, notochordal cells which play an important role in maintaining disc integrity are present in the NP up to approximately 10 years of age, while in bovines, little if any notochordal cells are present by the time they are born. Furthermore, the bovine coccygeal IVDs are subject to lower physiological loads than human lumbar discs during their shorter lifetime.

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