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Purpose:
B. Poulet

MOUSE ANKLE JOINTS ARE, UNLIKE KNEES, RESISTANT TO LOAD-INDUCED LESION FORMATION
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Purpose: Incidence of osteoarthritis (OA) in human ankle joints has been
reported to be much lower than in the knee. In addition, ankle cartilage
has been shown to be relatively resistant to damage from mechanical
and biochemical insults compared to the knee joint. The mechanisms involved in
this relative protection of ankle cartilage are, however, still unknown.
Development of an animal model for studying the effects of mechanical
loading in the ankle joint would be very valuable in helping to define
those mechanisms. Herein, we exploited a model used for tibia and knee
joint loading in a mouse, in which localised load-induced femoral cartilage
lesions are formed, to determine whether it may also serve as a model to
study this relative protection of ankle joints.

Methods: The right tibia of 8 week-old CBA mice were loaded 3 times
a week for 2 weeks as described previously. Ankle joints were dissected,
embedded and sectioned (sagittal, 9µm). Toluidine blue stained sections
at regular interval across the ankle joint were scored for articular
cartilage (AC) lesions (grade 0-4; 0=normal; 1=loss of stain; 2=lesion in
uncalcified cartilage; 3=lesion in calcified cartilage; 4=exposed subchon-
dral bone). Maximum scores across the tibia-talus, talus-calcaneum and
calcaneum-navicular joints were used and statistical analysis was performed
by non-parametric Mann-Whitney test. Loss of proteoglycans was also visualised with Safranin O staining. Abnormalities were also noted for
non-cartilaginous tissues.

Results: Unlike knee joints, ankle joints in CBA mice did not show any
significant changes in AC lesion severity with loading (1.2±0.37 right
loaded ankles and 1.37±0.37 in contra-lateral non-loaded control joints). Safranin O, as a measure of PG loss, was well distributed across the
uncalculated cartilage and decreased in the calcified cartilage in control
joints. Loading did not affect this staining. The most consistent changes in
response to loading were seen at the insertion of the Achilles tendon into
the calcaneum: this showed localised cell hypertrophy and an elaboration of a PG-rich matrix. Loaded ankle joints in some animals also developed
synovial activation between the calcaneum and talus bones.

Conclusions: Loaded ankles showed no AC pathological responses. Compar-
ison with loaded knee joints in the same limbs demonstrated a protection
from load-induced lesion formation. Ankle joints did, however, develop some histological changes in tendon structure at the calcaneal insertion and
some synovial activation. Similar, but more severe, changes in equivalent
structures were also observed in response to loading in the knee joint.
Our data indicate that this murine in vivo loading model shows a relative
protection of ankle AC to mechanical damage. Use of this model will
allow direct comparison of knee and ankle joints in response to loading
in the same limb and highlight the potential for its use in examining the
importance of specific genes in the relative protection of ankle joints to
mechanical insult.

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IDENTIFICATION OF JOINT OCHRONOSIS IN A MOUSE MODEL OF ALKAPTONURIA
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Purpose: Alkaptonuria (AKU) is a rare genetic disease resulting from
deficiency of the enzyme homogentisate 1,2-dioxogenase (HGD). It is char-
acterized by elevated circulation of homogentisic acid (HGA), a metabolite
that is deposited as a dark polymer, termed ochronotic pigment, in coll
laneous tissues particularly the articular cartilages of the weight bearing
joints. Ochronosis leads to a severe, early onset osteoarthropathy, which
currently has no known treatment. One of the limitations in studying
ochronosis is the lack of experimental models. Mice deficient in HGD exist
but do not normally develop ochronosis, despite elevated circulating and
urinary HGA. We recently detected deposition of ochronotic pigment in the
kidneys of mice, heterozygous for the AKU mutation and homozygous for
fumaryl acetoacetate hydratase (FAH), the enzyme absent in tyrosinemia
type I. These animals are usually maintained on nitisinone, but some
survive withdrawal of the drug. The aim of this study was to determine if
these mice develop joint ochronosis.

Methods: 17th month old C57 mice (AKU+/−, FAH−/−) were withdrawn
from nitisinone for 1months and sacrificed for study. Animals were
dissected and joint tissues and organs processed for routine histology.
Tissues were stained with Schmorlos reagent which is a sensitive stain for
ochronotic pigment.

Results: Macroscopic investigation of the renal system revealed pigmented
nodules in the kidney. Microscopy confirmed these to be ochronotic pig-
ment. Initial inspection of the joints did not show gross pigmentation or
evidence of joint degeneration. However, careful histological analysis
identified ochronotic pigment in the articular cartilages of the knee joints.
Chondrocytes in the calcified cartilage of the distal femur, proximal tibia
and fibula all displayed pigment in the pericellular and territorial matrix.
Pigmentation was not seen in the bone matrix, or other cartilage zones.
Staining with Schmorlos reagent confirmed the presence of ochronotic
pigment in these regions, consistent with findings in human AKU tissues.

Conclusions: Here we describe the first observations of ochronosis in
joint tissues of mice. The pattern of initial pigmentation appears to be
remarkably similar to that in human AKU, indicating that this will be an
excellent model to investigate the initiation and progression of ochronosis.
It had previously been thought that animals with the AKU mutation did
not display any of the phenotype observed in humans. Several theories
have been advanced for the lack of ochronosis, including insufficient life
span; failure to reach threshold circulating levels of HGA and/or the
protective effects of ascorbic acid, which in mice is an endogenously
produced anti-oxidant that inhibits HGA polymerisation and protects joint
matrix components. The AKU+/−, FAH−/−, mice are normally maintained
on nitisinone but some mice can adapt to long term withdrawal, undergoing
spontaneous, clonal loss of heterozygosity for the AKU mutation. The
subsequent increase in HGA coupled with deterioration of renal function
appears to trigger pigment deposition in the joints of the mice. We believe
these observations will provide a useful model to investigate ochronosis and
to develop therapeutic strategies for prevention of joint destruction in AKU.
This model should also be useful to investigate pathogenesis of generalized
osteoarthropathy.