The development of RNA extraction protocols to examine the effects of early exercise on gene expression in the articular cartilage and subchondral bone of Perendale sheep

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ABBREVIATIONS

AC	Articular cartilage
ADAM TS	Disintegrin matrix metalloproteinases with thrombospondin repeats
ADAMS	Disintegrin matrix metalloproteinases
BMD	Bone mineral density
BMP	Bone morphogenic protein
CT	Computed tomography
DJD	Degenerative joint disease
DXA	X-ray absorptiometry dual energy
GAPDH	Glyceraldehyde phosphate dehydrogenase
IL-1β	Interleukin-1-beta
MMP	Matrix metalloproteinase
MRI	Magnetic resonance imaging
OA	Osteoarthritis
OCD	Osteochondrosis dissecans
PCR	Polymerase chain reaction
PRF	Peptide regulatory factors
RA	Rheumatoid arthritis
RER	Rough endoplasmic reticulum
RT	Reverse transcription
Rt	Real time
SCB	Subchondral bone
TIMP	Tissue inhibitor metalloproteinase
TNF-α	Tumour necrosis factor alpha
US	Ultrasonography
VEGF	Vascular endothelial growth factors
β-Actin	Beta actin

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ABSTRACT

To examine gene expression *in vivo*, total RNA was extracted from articular cartilage and subchondral bone. As limited methodology existed for ovine, RNA extraction was performed by optimization of previously published protocols used for other species and *in vitro* studies (Chomczynski & Mackey, 1995; Chomczynski & Sacchi, 1987; Heinrichs et al., 1997). Reverse transcription polymerase chain reaction (RT-PCR) was used to amplify the extracted RNA to evaluate the gene expression of inflammatory cytokines, collagens, collagenase and housekeeping genes glyceraldehyde phosphate dehydrogenase and Beta-Actin. We observed changes in the expression of inflammatory cytokines, collagen and collagenase genes between exercised and unexercised sheep. The results of this research are consistent with clinical imaging and microscopy studies which suggest that moderate exercise during early life can stimulate an adaptive response in articular cartilage and subchondral bone (Brama, Tekoppele, Bank, Barneveld, & van Weeren, 2000; Firth, 2006; Firth & Rogers, 2005b; Lammi et al., 1993). These changes can have a chondro-protective effect (Jones, Bennell, & Cicuttini, 2003; Otterness et al., 1998) and may reduce susceptibility to athletic injury in later life. Future research using fluorescent probes and polymerase chain reaction may permit quantification of gene expression in real-time to determine the anabolic and catabolic response of articular cartilage with developmental age and exercise *in vivo*.