**EFFECTS OF TOPICAL GLYCERYL TRINITRATE ON FEMORO-TIBIAL ARTICULAR CARTILAGE AND SUBCHONDRAL BONE IN NORMAL AND OVARIECTOMIZED SHEEP**

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**Introduction:** The effect of nitric oxide radical (NO) on articular cartilage (AC) is currently unclear, with experimental evidence of both deleterious and chondroprotective roles. NO has been shown to suppress proteoglycan (PG) synthesis [1] and bone studies suggest that NO may be an important inhibitor of osteoclastic resorption [2]. Given *in vivo* evidence that NO promotes the progression of arthritic changes, the safety of NO donor compounds, widely used clinically for the treatment of hypertension and angina, may be questioned. The aim of this study was to examine the effects of glyceryl trinitrate (GTN), an exogenous nitric oxide donor, on the structural and biomechanical integrity of normal articular cartilage and subchondral bone in an aged ewe model. In addition, as oestrogen status may modify NO responses [3], the effects of GTN were also examined in ewes subjected to ovariectomy to deplete their circulating oestrogen levels.

**Methods:** Twenty four aged (7 year old) ewes were used for this study. 12 were ovariectomised by midline laparotomy. Half were untreated (OVX), while the remainder were treated topically with 2% GTN ointment (0.7 mg/kg) [Nitrobid®, Hoescht, Australia] twice per week (OVX+GTN). Of the 12 non-operated sheep, half were treated with the same dose of GTN (NOC+GTN) and the remaining six used as normal controls (NOC). All animal procedures were approved by the Murdoch University Animal Ethics Committee. After sacrifice at 26 weeks, dynamic biomechanical testing of tibial plateau cartilage was conducted using a novel hand-held practice.

**Conclusion:** NO donation via GTN treatment reduced the thickness and phase lag of femoro-tibial UCC. Ovariectomy produced similar changes including increased superficial CB indicative of reorganised collagen assembly. Reduction of tibial AC phase lag and toludine blue staining of femoral condyle AC suggest a disturbance of proteoglycan metabolism[6]. This is consistent with the known involvement of NO in interleukin-[β]-induced suppression of PG synthesis, chondrocyte apoptosis, and hyporesponsiveness to insulin-like growth factor-1 (IGF-1) in arthritic cartilage. GTN also caused an increase in SCB thickness in the MTP consistent with the bone-promoting actions of NO. However, GTN appeared to have an opposite effect on phase and UCC thickness changes in OVX+GTN animals. By preventing post-ovariectomy subchondral bone loss, GTN may have had a ‘stabilising’ effect on cartilage structure. Alternatively, this may relate to the combined effects of GTN-associated proteoglycan changes and post-ovariectomy collagen disruption [7], leading to cartilage swelling. These results lead us to suggest that prolonged clinical use of nitrates, such as GTN, may be detrimental to articular cartilage, and that such effects may be modified by menopausal status. This highlights the need for further epidemiological evaluation of the safety of nitrates such as GTN in clinical practice.

| Table 1: Cartilage PG content, as measured by intensity of toluidine blue staining (grayscale pixel intensity, black=255) *p<0.05 (vs NOC) |
| NOC | NOC+GTN | OVX | OVX+GTN |
| MFC | 152.4±7.0 | 146.3±5.7* | 145.0±3.5* | 141.5±4.4* |
| LFC | 148.0±5.5 | 145.4±6.1 | 138.5±2.5* | 142.1±3.8* |

**Figure 2:** Mean uncalcified cartilage (above) and subchondral bone plate (below) thickness (mm ± SE).


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