EFFECTS OF HYPOXIA ON CELL PROLIFERATION AND MATRIX SYNTHESIS IN SYNOVIUM AND TENDON FROM THE RABBIT CARPAL TUNNEL

**Introduction:** Morphologic changes consistent with ischemia (e.g., edema, fibrous hyperplasia, and perivascular thickening) are observed in synovium early in carpal tunnel syndrome (Schuind 90). A role for ischemia in the pathophysiology is also suggested by the elevated hydrostatic pressure within the tunnel observed in patients with carpal tunnel syndrome in comparison to healthy controls (Rojviroj 90). How synovium and tendon cells react to ischemia or the associated hypoxia is not known. The purpose of this study was to examine, in vitro, the effects of oxygen tension on cell proliferation and the synthesis of extracellular matrix macromolecules in synovial and tendon explants from the rabbit carpal tunnel.

**METHODS**
Twelve mixed gender Swedish Loop rabbits were used. Using a dissecting microscope, synovial and tendon tissue from the carpal tunnel was dissected free from adjacent tissues and the FDP tendon removed. The synovial tissue from each paw was split once longitudinally to produce 4 explants per animal. The FDP tendon was cut into two 1 mm thick cross-sectional slices also producing 4 explants per animal. The tissues were divided to 4 culture trays. The study was approved by the Lund University Committee on Animal Research.

Explants were cultured for three days. During the second and third days, the explants were incubated in airtight containers flushed with either 0, 1, 3, or 20% O2 plus 2.0 % CO2 and N2 to balance. Oxygen levels were measured at each tray. Twelve mixed gender Swedish Loop rabbits were used. Using a dissecting microscope, synovial and tendon tissue from the carpal tunnel was dissected free from adjacent tissues and the FDP tendon removed. The synovial tissue from each paw was split once longitudinally to produce 4 explants per animal. The FDP tendon was cut into two 1 mm thick cross-sectional slices also producing 4 explants per animal. The tissues were divided to 4 culture trays. The study was approved by the Lund University Committee on Animal Research.

**RESULTS**
Cell proliferation was assessed by uptake of 3H-thymidine (Fig 1). The synovium and tendon response to oxygen differed (p=0.03). For synovium the uptake was 25.3 (±0.1) x 10^3 dpm/mg in 20% O2 and was significantly greater than that in 0% O2 (0.3 (±0.8) x 10^3 dpm/mg). The uptake in tendon was 6.8 (±0.4) x 10^3 dpm/mg in 20% O2 and was significantly reduced at the lower O2 levels. The uptake in tendon was 0.6 (±0.1) x 10^3 dpm/mg in 20% O2 and was also significantly reduced at the lower O2 levels.

The synthesis of collagen was assessed by uptake of 3H-Hydroxyproline. The response of synovium and tendon to oxygen did not differ (p=0.77). The uptake in synovium explants was 0.6 (±0.1) x 10^3 dpm/mg in 20% O2 and was significantly reduced at the lower O2 levels. The uptake in tendon was 8.9 (±0.5) x 10^3 dpm/mg in 20% O2 and was also significantly reduced at the lower O2 levels.

DISCUSSION
Synovial tissue from the rabbit carpal tunnel demonstrated a reduction in cell proliferation and an alteration in the synthesis of matrix proteins with exposure to low oxygen levels. Cell proliferation dropped by two-thirds when oxygen level decreased. Similarly, the synthesis of collagen and non-collagen proteins dropped by half. On the other hand, the synthesis of proteoglycans was not altered by oxygen tension. Therefore, the relative quantity of proteoglycan synthesized was increased at the low oxygen tensions. In contrast, tendon was either much less sensitive or not sensitive at all to the effects of hypoxia.

It is not likely that the synovium and flexor tendons from the carpal tunnel are exposed to static oxygen levels, as used in this study, but are exposed to fluctuating levels depending on local tissue hydrostatic pressures which are influenced by hand postures (Rempel 97). Unfortunately, the oxygen levels in these tissues are unknown. However, the relevance of testing the low oxygen levels was demonstrated by Takemiy a et al. (88). Using an oxygen electrode, in vivo recordings from the rabbit Achilles tendon revealed PO2 levels of 4.1 ± 2.0 kPa. Our results demonstrate that hypoxia can inhibit synovial cell proliferation and alter the synthesis of matrix macromolecules and suggests a possible mechanism for initial cellular damage at low oxygen levels. Such injury may be followed by edema and tissue repair responses which elevate carpal tunnel pressure leading to ischemic effects on adjacent tissues, e.g. the median nerve.

**References.**

**Acknowledgements.** Fogarty International Center/NIH (F06 TW02250-01) and Swedish Medical Research Council (B96-17X05948-16B)

**First Name Last Name**

*Rempel, D., +Abrahamsson, S.-O., *Dept. of Medicine, University of California San Francisco, 1301 S 46th St, Bldg 112, Richmond, CA 94804, (510)231-5720, FAX (510)231-5729, rempel@ms.ucsf.edu, +Dept. of Hand Surgery, Malmö University Hospital, 20502 Malmö, Sweden, (46)40-331725, FAX:6640-928855, sven.abrahamsson@hand.mas.lu.se

*First Name Last Name*

PC 6/7 ABSTRACT NO. 1568

**PRESENTING AUTHOR:** David Rempel

**CORRESPONDING AUTHOR:** David Rempel

**The synthesis of collagen was assessed by uptake of 3H-Hydroxyproline. The response of synovium and tendon to oxygen did not differ (p=0.77). The uptake in synovium explants was 0.6 (±0.1) x 10^3 dpm/mg in 20% O2 and was significantly reduced at the lower O2 levels. The uptake in tendon was 0.8 (±0.4) x 10^3 dpm/mg in 20% O2 and was also significantly reduced at the lower O2 levels.**

**Oxygen level (kPa)**

```
<table>
<thead>
<tr>
<th>Oxygen level (kPa)</th>
<th>0</th>
<th>5</th>
<th>10</th>
<th>15</th>
<th>20</th>
</tr>
</thead>
<tbody>
<tr>
<td>Synovium</td>
<td>0.6 (±0.1) x 10^3 dpm/mg</td>
<td>0.8 (±0.4) x 10^3 dpm/mg</td>
<td>8.9 (±0.5) x 10^3 dpm/mg</td>
<td>0.6 (±0.1) x 10^3 dpm/mg</td>
<td>0.8 (±0.4) x 10^3 dpm/mg</td>
</tr>
<tr>
<td>Tendon</td>
<td>6.8 (±0.4) x 10^3 dpm/mg</td>
<td>0.6 (±0.1) x 10^3 dpm/mg</td>
<td>0.8 (±0.4) x 10^3 dpm/mg</td>
<td>7.6 (±0.3) x 10^3 dpm/mg</td>
<td>0.8 (±0.4) x 10^3 dpm/mg</td>
</tr>
</tbody>
</table>
```

Figure 1. Effect of oxygen on cell proliferation in synovium (+ - +) and tendon (- - +). 3H-Thymidine mean values are dpm/mg dwt x 10^3 (±SEM).

**DISCUSSION**
Synovial tissue from the rabbit carpal tunnel demonstrated a reduction in cell proliferation and an alteration in the synthesis of matrix proteins with exposure to low oxygen levels. Cell proliferation dropped by two-thirds when oxygen level decreased. Similarly, the synthesis of collagen and non-collagen proteins dropped by half. On the other hand, the synthesis of proteoglycans was not altered by oxygen tension. Therefore, the relative quantity of proteoglycan synthesized was increased at the low oxygen tensions. In contrast, tendon was either much less sensitive or not sensitive at all to the effects of hypoxia.

It is not likely that the synovium and flexor tendons from the carpal tunnel are exposed to static oxygen levels, as used in this study, but are exposed to fluctuating levels depending on local tissue hydrostatic pressures which are influenced by hand postures (Rempel 97). Unfortunately, the oxygen levels in these tissues are unknown. However, the relevance of testing the low oxygen levels was demonstrated by Takemiy a et al. (88). Using an oxygen electrode, in vivo recordings from the rabbit Achilles tendon revealed PO2 levels of 4.1 ± 2.0 kPa. Our results demonstrate that hypoxia can inhibit synovial cell proliferation and alter the synthesis of matrix macromolecules and suggests a possible mechanism for initial cellular damage at low oxygen levels. Such injury may be followed by edema and tissue repair responses which elevate carpal tunnel pressure leading to ischemic effects on adjacent tissues, e.g. the median nerve.

**References.**

**Acknowledgements.** Fogarty International Center/NIH (F06 TW02250-01) and Swedish Medical Research Council (B96-17X05948-16B).