

## Supplementary Data File 6.

### Changes in response to Electrical Field Stimulation caused by application of L-NAME or atropine

#### Methods

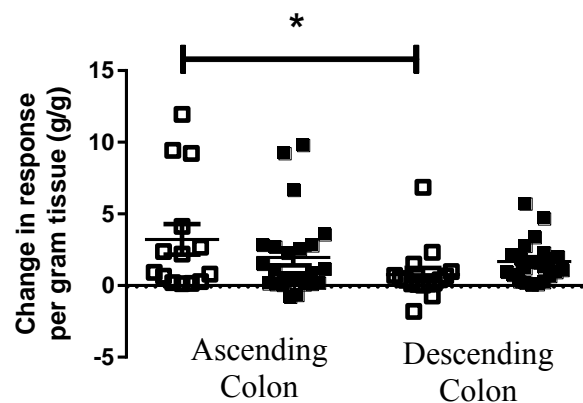
After approval by the local ethics committee (REC 10/H0703/71), written informed consent was obtained for use of macroscopically-normal ascending and descending/sigmoid (referred to hereon as descending) colon (5-10 cm from tumour) from patients undergoing elective surgery for non-obstructing bowel cancer. Patient records were examined for use of ongoing medication and evidence of comorbidity but a systematic survey of bowel functions (e.g. stool output/frequency) was not conducted. No patient had previous chemo- or radiotherapy or a diagnosis of inflammatory bowel disease. The time between surgery and immersion of tissue into pre-oxygenated Krebs solution was between 60 and 120 min. Tissues were separated into two discontinuous age groups: Adult (35-60 years) and Elderly ( $\geq 70$  years).

After removing the mucosa, strips were cut parallel to the circular muscle (~5mm wide, 10-15mm long; 3-31 from each patient) and mounted in warmed tissue baths containing Krebs solution (mmolL<sup>-1</sup>: NaCl 121.5, CaCl<sub>2</sub> 2.5, KH<sub>2</sub>PO<sub>4</sub> 1.2, KCl 4.7, MgSO<sub>4</sub> 1.2, NaHCO<sub>3</sub> 25, glucose 5.6, equilibrated with 5% CO<sub>2</sub>/95% O<sub>2</sub>) for measuring isometric muscle tension. Electrical field stimulation (EFS) was applied at 5 Hz and at 50V for 10s every 1 min to evoke neuronally-mediated responses (sensitive to prevention by tetrodotoxin 1  $\mu$ M) in ascending and descending colon from adult (35 to 60 years) and elderly ( $\geq 70$  years) patients.

#### Results

The figure shows the change in response during EFS after application of L-NAME 300  $\mu$ M, in each region of adult (35 - 60 years of age;  $\square$ ) and elderly ( $\geq 70$  years;  $\blacksquare$ ) colon, for each tissue tested. The data are expressed as the change in g tension/ g wet weight of tissue, generated during EFS, together with the mean  $\pm$  S.E.M. contractile force. Respectively, N = 14, 25, 16 and 23 in adult ascending, elderly ascending, adult descending and elderly descending colon. \* represents P < 0.05 only where indicated.

Change in response during EFS following application of L-NAME 300  $\mu$ M



## Conclusion

The cholinergically-mediated contraction evoked during EFS, particularly in ascending colon, is negatively modulated by simultaneous activation of inhibitory nitreergic neurons.