

10.1302/2046-3758.136.BJR-2023-0360.R1

Table i. Primers used for gene expression analysis.

Gene	QuantiTect Primer Assay (Qiagen)
<i>Acan</i>	QT00189518
<i>Acta2</i>	QT01615901
<i>Arg1</i>	QT00177611
<i>Ccn2</i>	QT00182021
<i>Col1a1</i>	QT00366016
<i>Col2a1</i>	QT01084118
<i>Col3a1</i>	QT00365981
<i>Eln</i>	QT00413007
<i fn1<="" i=""></i>	QT00179333
<i>Hprt1</i>	QT00199640
<i>MMP9</i>	QT00178290
<i>MMP13</i>	QT01629593
<i>Nfkb1</i>	QT00370545
<i>Nos2</i>	QT00178325
<i>Tgfb1</i>	QT00187796
<i>Timp1</i>	QT00185304

Acan, aggrecan; *Acta2*, actin alpha 2, smooth muscle; *Arg1*, arginase 1; *Ccn2*, cellular communication network factor 2; *Col1a1*, collagen type I alpha 1 chain; *Col2a1*, collagen type II alpha 1 chain; *Col3a1*, collagen type III alpha 1 chain; *Eln*, elastin; *Fn1*, fibronectin 1; *Hprt1*, hypoxanthine phosphoribosyltransferase 1 (housekeeping gene); *MMP9*, matrix metalloproteinase 9; *MMP13*, matrix metalloproteinase 13; *Nfkb1*, nuclear factor kappa B subunit 1; *Nos2*, nitric oxide synthase 2; *Tgfb1*, transforming growth factor, beta 1; *Timp1*, TIMP metalloproteinase inhibitor 1.

Table ii. Surgery details and postoperative weight loss.

Variable	Male		p-value*	Female		p-value*
	Saline (n = 10)	ALM (n = 11)		Saline (n = 10)	ALM (n = 11)	
ACL rupture profile, n						
Complete (partial)	9 (1)	9 (2)	> 0.999	9 (1)	9 (2)	> 0.999
Proximal (mid-substance)	10 (0)	10 (1)	> 0.999	9 (1)	11 (0)	> 0.999
Mean operating time, mins (SD)†	47.8 (7.3)	49.3 (8.1)	> 0.999	49.4 (7.1)	47.1 (4.9)	> 0.999
Mean blood loss, g (SD)	1.25 (0.62)	1.1 (0.34)	> 0.999	0.64‡ (0.25)	0.69 (0.33)	> 0.999

*Kruskal–Wallis with Dunn’s post-hoc test.

†Inclusive of catheter insertion, tendon harvest, ACL reconstruction surgery, and catheter removal.

‡p < 0.05, Saline female compared to Saline male.

ACL, anterior cruciate ligament; ALM, adenosine, lidocaine and magnesium therapy; SD, standard deviation.

Table iii. Comparison of hind limb ventral temporal-spatial gait parameters for adenosine, lidocaine, and magnesium-treated and Saline control male and female animals prior to (day -1) and following (day 28) anterior cruciate ligament reconstruction surgery. All values are expressed as means and standard errors.

Indices	Sex	Tx group	Time post-ACLR surgery	
			Day -1	Day 28
Stance width, cm	Male	Saline	5.1 (0.2)**	5.8 (0.2)‡***
		ALM	5.2 (0.1)§	6.1 (0.2)‡§
	Female	Saline	4.4 (0.1)	4.5 (0.1)
		ALM	4.3 (0.1)	4.8 (0.1)‡
Stride length,* cm	Male	Saline	18.1 (0.3)**	17.6 (0.7)
		ALM	17.0 (0.3)	17.8 (0.7)
	Female	Saline	15.8 (0.4)	16.5 (0.7)
		ALM	17.4 (0.4)	18.9 (0.6)†‡
Stride length variability,* %	Male	Saline	6.8 (0.7)	13.5 (1.6)
		ALM	8.2 (0.9)	12.6 (1.1)
	Female	Saline	10.1 (1.1)	20.1 (7.4)‡
		ALM	11.6 (2.7)	10.1 (1.3)
Stride time,* s	Male	Saline	0.43 (0.02)	0.50 (0.04)
		ALM	0.43 (0.01)	0.46 (0.03)¶
	Female	Saline	0.39 (0.02)	0.40 (0.03)
		ALM	0.37 (0.02)	0.35 (0.03)
Stride time variability,* %	Male	Saline	21.2 (2.9)	25.8 (5.3)
		ALM	24.6 (3.8)	27.3 (2.7)
	Female	Saline	25.3 (4.1)	21.1 (3.5)
		ALM	19.2 (2.8)	18.8 (3.6)
Step length, cm	Male	Saline	10.3 (0.2)**	10.5 (0.2)**
		ALM	9.9 (0.1)	10.6 (0.3)‡§
	Female	Saline	9.1 (0.2)	9.4 (0.3)
		ALM	9.7 (0.1)	10.5 (0.3)†‡
Paw angle,* °	Male	Saline	0.4 (0.2)	0.5 (0.1)
		ALM	0.3 (0.2)	0.5 (0.1)
	Female	Saline	0.2 (0.3)	0.8 (0.3)‡
		ALM	0.5 (0.1)	0.5 (0.1)
Track speed, cm/s	Male	Saline	43.2 (2.0)	38.1 (3.9)
		ALM	40.6 (1.9)	39.8 (3.7)¶
	Female	Saline	42.1 (2.6)	44.6 (5.0)
		ALM	49.6 (4.1)	58.2 (5.2)

Animals per treatment group, per sex: Saline, n = 10; ALM, n = 11.

p-values were calculated using mixed analysis of variance, with Tukey's post-hoc test.

*Operated (right) hind limb values.

†p < 0.05 ALM compared to Saline.

‡p < 0.05 compared to day -1.

§p < 0.05 ALM male compared to Saline female.

¶p < 0.05 ALM male compared to ALM female.

**p < 0.05 Saline male compared to Saline female.

ALM, adenosine, lidocaine, and magnesium therapy.

A



B

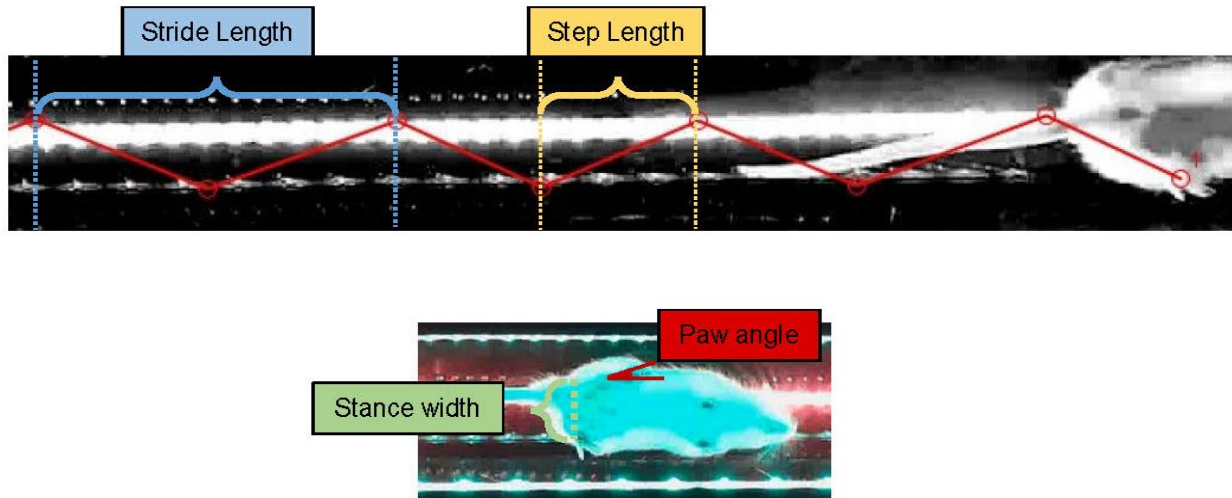


Fig a. a) Gait testing apparatus. A three-sided, custom catwalk was fabricated from 3 mm thick acrylic panels to the following specifications: 1.2 m × 12 cm × 10 cm (length × width × height). The catwalk was fixed to a clear glass surface, with a camera (Nexigo, N980P) positioned at a distance of 46 cm below the glass, and a darkened chamber placed at the end of the catwalk. To provide contrast for footpad contact and segmentation analysis, green (25% brightness) and red (50% brightness) LED strip lights were fixed to the inner surfaces of the catwalk. b) Gait parameters assessed included stride and step length, stance width, and paw angle.

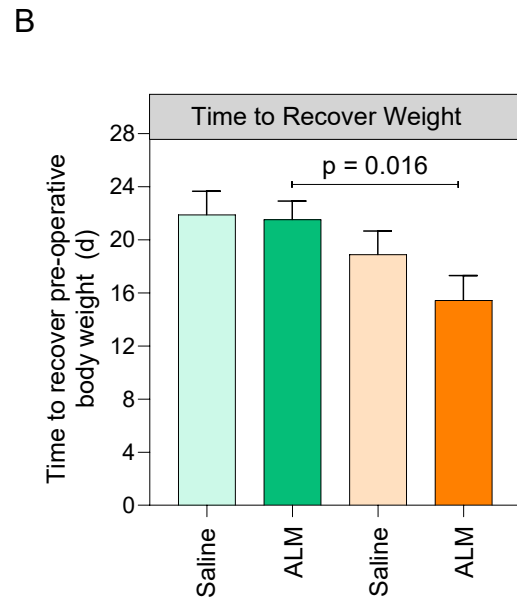
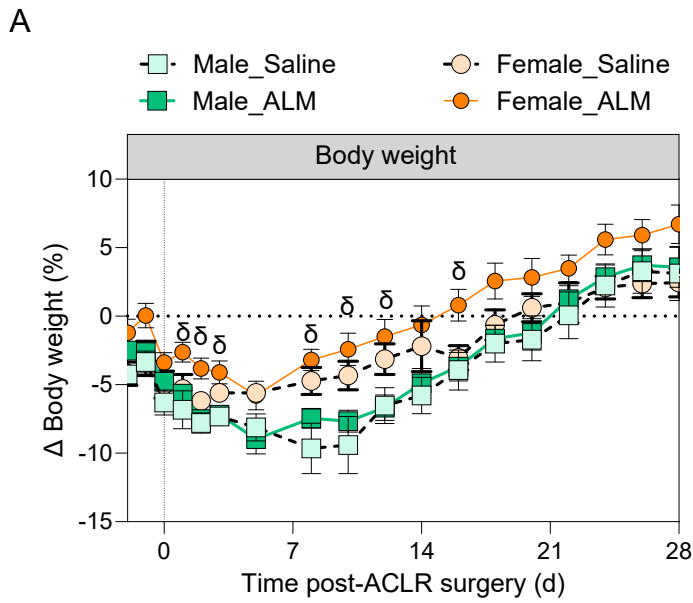


Fig b. Recovery of total body weight following anterior cruciate ligament (ACL) rupture (day -3) and ACL reconstruction (ACLR) surgery (day 0). a) Percentage change in body weight, and b) the time taken to recover preoperative body weight for adenosine, lidocaine, and magnesium (ALM)-treated and Saline control male and female animals following ACLR surgery. Data show mean and standard error. Mixed analysis of variance, Tukey's multiple comparisons test. $\delta p < 0.05$, ALM male compared to ALM female.