IN VIVO INHIBITION OF NITRIC OXIDE SYNTHASE IN OVINE MUSCLE INCREASES POST-SLAUGHTER LACTATE PRODUCTION AND IMPROVES MEAT TENDERNESS.

J.J. Cottrell[1,2], F.R. Dunshea[2], M.B. M[c]Donagh[2], R.D. Warner[1,2]
[1] School of Life Sciences and Technology, Victoria University, PO 8001 MCMC, Victoria, Australia.
[2]Natural Resources and Environment, 600 Sneydes Rd, Werribee Victoria 3030, Australia.

Background

Nitric oxide (NO) is a free radical synthesised intracellularly during the conversion of L-arginine to L-citrulline by nitric oxide synthase (NOS). NO diffuses intracellularly and possesses high binding affinities to cellular heme and thiols, modulating the function of many cellular proteins. NOS activity is increased by muscular activity (Roberts *et al.*, 1999) where it decreases the force of contraction (King-Vanvlack *et al.*, 1995), inhibits cellular respiration (Young *et al*, 1997) and calcium release (Hart and Dalhunty, 2000) in what may be a protective function during exercise, or part of muscular fatigue. Understanding NO metabolism provides new possibilities for controlling and improving physiological aspects of meat science, particularly pre-slaughter stress. Factors affecting fresh meat quality such as energy and calcium metabolism, proteolysis, fibre type, sarcomere length, stress and age all interact with NO messaging systems or nitric oxide synthase (NOS) expression. Investigation into the role of NO in fresh meat quality provides new avenues for a better understanding of how pre-slaughter physiology influences meat quality.

Objectives

To determine the effect of changing NOS activity with L-NAME infusion and exercise on plasma and muscle lactate, pH and meat tenderness.

Methodology

Forty Border Leicester cross Merino lambs, approximately six months old (33.5 to 51kg live weight) were acclimatised in individual pens for 10-14 days pre-slaughter with *ad libitum* access to lucerne chaff, lamb cubes (15% crude protein, 12% MJ ME/kg dry matter) and water. An indwelling jugular catheter (12 Gauge) was inserted 1-2 days pre-slaughter. Lambs were deprived from food for approximately 12 hours pre-slaughter, but had *ad libitum* access to water. Treatments were applied using a 2x2 factorial with a balanced randomised block design. Lambs were infused intravenously with 10mL of saline (0.9% NaCl) or saline and 30 mg/kg L-arginine methyl ester hydrochloride (L-NAME) 135 minutes pre-slaughter. At 120 min, lambs were moved to the abattoir (approximately 150m) either with a small flock of trained sheep or individually exercised for 15 minutes in a paddock. Immediately pre-slaughter blood samples were obtained from all lambs. Muscle samples were removed from the *Longissimus thoracis et lumborum* (LTL) (2 min, 6 and 24 h) and *Semimembranosous* (SM) (2 & 30 min, 1, 2, 4, 6 and 24h) post-slaughter for determination of muscle lactate. Muscle pH and temperature were measured at 30 min and 24h post-slaughter and tenderness measured by Warner Bratzler shear force (WBSF) at 1 and 3 days of ageing in a vacuum bag and storage at 4°C. All data were tested for significance with an analysis of variance (ANOVA).

Results and Discussion

L-NAME did not effect plasma lactate concentrations pre-slaughter (Table 1) but increased lactate content in exercised but not non-exercised LTL and SM muscles post-slaughter (Figures 1 and 2). NO inhibits cellular metabolism, through inhibition of enzymes such as cytochrome c oxidase (Cleeter *et al.*, 1994) and glyceraldehyde-3-phosphate (Dimmeler *et al.*, 1992). These data show that inhibition of NOS with L-NAME antagonises NO mediated inhibition of cellular metabolism, resulting in increased muscle lactate content. Since L-NAME was effective in increasing muscle lactate production in exercised but not non-exercised lambs the data suggests that NOS activity is increased by exercise. Muscular activity is associated with a variety of stressors in commercial settings including mixing and cold stress, fright and transport. Our results indicate increased NOS activity with pre-slaughter stress and providing new knowledge to better understand sub-cellular pathways that predispose livestock to negative meat quality traits such as dark-firm-dry (DFD) and pale-soft-exudative (PSE) meat.

Increased lactate production had ceased by 6 hours in both muscles and did not affect pH at 30 min or 24h post-slaughter. It is likely that NOS activity is attenuated post-mortem due to a lack of O_2 and NADPH as substrates (Marletta, 1993). Therefore, increased lactate production with L-NAME 2-4 hours post-mortem in exercised SM muscles is probably a result of putative downstream effects of NOS inhibition. Increases in exercised LTL and SM lactate production with L-NAME were not uniform. Lactate production was elevated immediately (5 min) post-slaughter in exercised LTL muscles, but not until 2 and 4 hours post-mortem in the SM. The LTL and SM have different anatomical roles, the LTL is primarily postural while the SM is used for locomotion (Totland and Kryvi, 1991). NOS expression is highly correlated to type II muscle fibres (Kobzik *et al.*, 1994) and the half life of NO varies considerably with cellular redox condition (eg anti-oxidants, free radicals, cellular thiol) and oxygen tension. Since the intracellular conditions of the LTL and SM muscles differ in resting and exercised states, it is likely that the half-life of NO and NOS activity will differ between the LTL and SM.

Different responses between the LTL and SM were also observed with meat tenderness measurements. WBSF was reduced (more tender meat) by L-NAME in the LTL with 3 days aging while no response was observed in the SM. NO has been demonstrated to reduce activity of calpain (Michetti *et al.*, 1995) and the ryanodine receptor (primary skeletal muscle Ca^{++} channel), providing potential mechanisms by which NO can influence meat tenderness.

Conclusion

Inhibition of NOS in lambs pre-slaughter has shown that NO dependant pathways contribute to post-slaughter ovine muscle metabolism. NOS inhibition only increased lactate production in exercised lambs, indicating that ovine LTL and SM NOS activity and muscle contractility are linked. It is known that many environmental stressors imposed on domestic livestock pre-slaughter influence muscular contractility and therefore elevate NOS activity. Since reducing NOS activity with L-NAME improved LTL tenderness, it is possible that NOS contributes to tougher meat associated with stressed livestock.

Table 1: Effects of L-NAME (none vs 30 mg/kg) and exercise (none vs. 15 min) on pre-slaughter plasma lactate and post-slaughter pH and WBSF of the LTL and SM muscles.

L-NAME		Control (saline)		L-NAME			F-value		
Exercise (EX)	Time post-slaughter	Control	Exercise	Control	Exercise	SED	L-NAME	EX	L-NAME x EX
Plasma lactate (mM)	0	2.9	11.1	2.1	11.0	1.96	0.75	<0.001	0.80
pH-LTL	30min	6.7	6.8	6.8	6.7	0.06	0.21	0.07	0.41
	1d	5.6	5.6	5.6	5.6	0.02	0.18	0.01	0.17
pH-SM	30min	6.8	6.7	6.8	6.7	0.05	0.93	0.001	0.65
	1d	5.5	5.5	5.5	5.5	0.04	0.69	0.60	0.38
WBSF-LTL (kg/cm^2)	1d	10.0	10.7	10.7	10.0	0.48	0.15	0.12	0.40
	3d	8.2	9.2	6.6	8.1	0.89	0.04	0.07	0.68
WBSF-SM (kg/cm^2)	1d	7.9	7.9	8.5	7.4	0.50	0.99	0.03	0.11
	3d	6.6	5.9	6.6	5.9	0.44	0.14	0.10	0.21

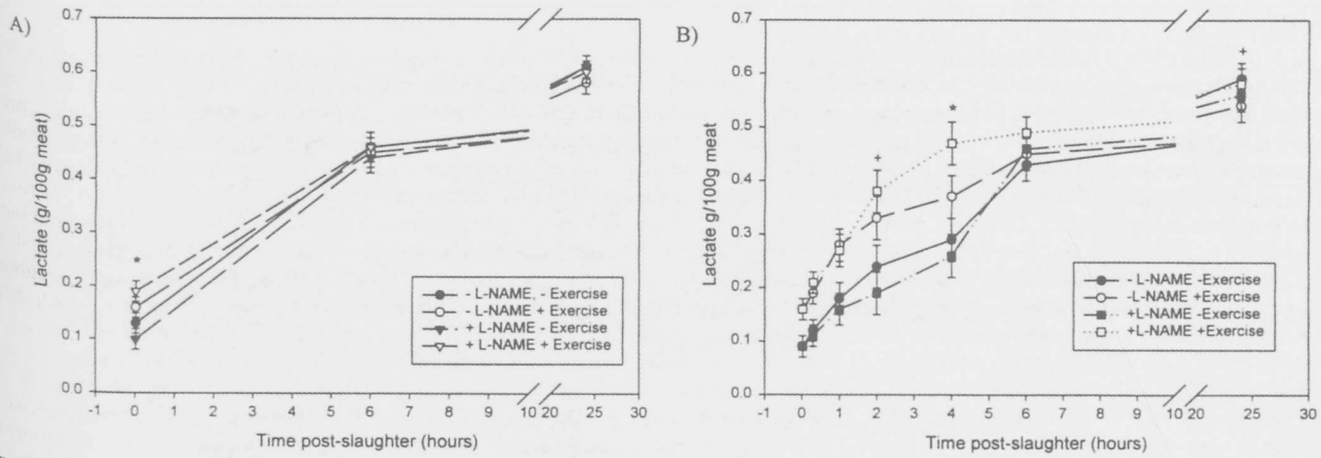

Figure 1: Effects of -L-NAME infusion (- vs. +) and exercise (- vs +) on A) LTL and B) SM lactate production post-slaughter. *, + denotes significance between means at a specific time point, $P<0.05$ and $P\leq0.10$ respectively.

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