Review

Effects of nitric oxide and oxidation in vivo and postmortem on meat tenderness

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Abstract

Metabolic processes in muscle tissue in vivo result in the production of reactive oxygen species and oxidative compounds including superoxide anions and nitric oxide (NO). Reactive oxygen species can react with both lipids and proteins and often have deleterious effects, contributing to the onset of ageing and senescence as well as cell death. Nitric oxide (NO) is a free radical that is constantly produced or released throughout the body by diverse tissues and is known to influence proteolytic activity in human and rodent skeletal muscle as well as being involved in regulation of calcium homeostasis in the muscle cell. The influence of nitric oxide on development of meat tenderness has been studied through postmortem manipulation and also through in vivo studies. The effect of NO on meat tenderness is postulated to be via its regulatory effects on the proteins calpain, cathepsins, ryanodine receptor channel in the sarcoplasmic reticulum (SR) and the sarcoplasmic–endoplasmic release calcium ATPase in the SR. NO is an oxidant although the effects of NO on effector proteins can be distinguished from a direct oxidation reaction. The onset of oxidation in meat postmortem is well known to produce off-odours, discoloration and unacceptable flavours associated with rancidity. Oxidation during the immediate postmortem period appears to inhibit tenderisation during ageing, probably through an inhibitory effect of oxidation on the calpain enzyme. Oxidation of muscle tissue occurring as a result of availability of oxygen during modified atmosphere packaging may also have deleterious consequences for tenderness development during storage of meat prior to retail display. In conclusion, it is proposed that postmortem meat tenderisation is influenced by skeletal muscle’s release of NO pre-slaughter and the oxidation of proteases postmortem. This proposal is compatible with the existing tenderness model and will hopefully assist in increasing the accuracy of prediction of meat tenderness. Future directions for research are discussed.

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Keywords: Nitric oxide; Oxidation; Tenderness; Calpain

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1. Introduction

The meat industry strives to deliver consistent, high quality products. Tenderness is a key eating quality attribute that the consumer expects yet a tenderness guarantee for beef, pork and lamb remains elusive. George, Tatum, Belk, and Smith (1999) reported that 72% of strip loins purchased from supermarkets in eight US cities were rated only “slightly tender” or “moderately tender”. Lack of consistent tenderness is a major quality problem that hinders the profitability of the beef (Morgan, 1995), lamb and pork industries. Experiments investigating meat tenderness often report large variations in tenderness between animals, kill days and abattoirs. With all the known factors included in a model for predicting tenderness there is still up to 50% of variation in tenderness unexplained. Animals vary in their response to any stressor and the stressors imposed on animals from farm to slaughter vary widely between days of kill and between abattoirs. It is our postulate that the “stress” physiology of any animal perimortem would be important in determining quality, through effects on ultimate pH, muscle glycogen and the dark-cutting condition but also through independent mechanisms. In a recent study in cattle, Warner, Ferguson, Cottrell, and Knee (2005) showed that the use of an electric prodder to induce acute stress 15 min pre-slaughter resulted in tougher beef loin which overall was less acceptable. The increase in toughness with the acute stress treatment was independent of rate of muscle pH or temperature decline, ultimate pH, sarcomere length or other known factors. The compound nitric oxide (NO), and its related molecules (S-nitrosothiols, metal NO complexes, higher oxides and some peroxynitrites), are involved in stress physiology and stress-related diseases (Esch, Stefano, Friccione, & Benson, 2002) and NO is also known to regulate calcium homeostasis in the muscle cell (Hare, 2003) and calpain activity (Michetti, Salamino, Melloni, & Pontremoli, 1995). This regulation is through redox-sensitive sites on calpain, the ryanodine receptor channel (RYR) (Hare, 2003) involved in calcium release from the sarcoplasmic reticulum (SR) and the sarcoplasmic-endo-sarcomplc reticulum calcium ATPase (SERCA) (Hare, 2003) involved in calcium uptake into the SR. Thus it is postulated that NO may influence meat tenderness postmortem either directly, through control of calpain activity, or indirectly through effects on calcium, as calcium is required for calpain activation (Dayton, Reville, Goll, & Stromer, 1976). This paper discusses recent results showing effects of NO on lamb and beef meat tenderness. This paper also discusses the potential role of oxidation of calpain early postmortem and also during storage in determining tender meat.

2. Oxidation and meat quality

In the instance of the live animal there is relatively low oxidative stress, and high anti-oxidant capacity. In terms of meat, and particularly the transition of muscle to meat, there is a high oxidant stress and relatively low antioxidant capacity to reverse the oxidative reactions. It is the difference between these two scenarios that makes oxidative stress important to meat quality. Oxidative stress in the living animal commonly results in reversible oxidation of the target protein. Redox signalling also entails at least one reaction which is reversible (Forman, Fukuto, & Torres, 2004). Halothane sensitive pigs are under sustained oxidative stress (Duthie, Arthur, Nicol, & Walker, 1989) and require higher levels of Vitamin E (Hoppe, Duthie, Arthur, Schoner, & Wiesche, 1989). Feeding Vitamin E to grain-fed beef cattle has consistently been shown to produce meat which has a longer display life, if all conditions (temperature, sanitation, etc.) are optimised, due to a delay in the onset of oxidation of oxymyoglobin to metmyoglobin (Mitsumoto, Arnold, Schaefer, & Cassens, 1993). Pasture fed cattle (Yang, Brewster, Lanari, & Tume, 2002) and lambs (Turner, McClure, Weiss, Borton, & Foster, 2002) usually have inherently high levels of Vitamin E in their musculature when grazing green pasture (Cetinkaya & Ozcan, 1991) due to high levels of β-carotene and Vitamin E in green pasture. Pro-oxidants accumulate in meat with time postmortem and contribute to an increasingly oxidative environment with time. Factors known to promote oxidation include availability...
of O₂, high temperatures, low pH, metal ions, particularly availability of iron in meat tissue, etc. Oxidative environments in meat post-slaughter are known to increase lipid oxidation, resulting in unfavourable consumer reaction to the flavour and smell due to onset of rancidity and also decrease shelf life, due to a rapid development of the brown pigment metmyoglobin. Oxidation can reversibly inactivate calpain in vivo (Guttmann & Johnson, 1998) and postmortem (Rowe, Maddock, Lonergan, & Huff-Lonergan, 2004b).

3. Nitric oxide production and oxidation in skeletal muscle in vivo

There is increasing evidence that, in addition to reactive oxygen species (ROS), muscle redox state is strongly influenced by NO (Barreiro, Gea, Corominas, & Hussain, 2003). Redox potential is defined as the state of oxidation of a system (EGHG, 1996) and also as a measure of the tendency of a system to donate or accept electrons, which is governed by the nature and proportions of the oxidizing and reducing substances contained in the system (WTI, 2005). Redox regulation in vivo has been perceived as a simple on-off switch for proteins involved in a range of metabolic roles (Kim et al., 2002) but in fact this is not the case. Cysteine residues seem to serve sensory and regulatory roles in the activation mechanism and confer responsiveness to nitric oxide and oxygen-related species and can differentiate nitrosative from oxidative events (Kim et al., 2002). Reduced thiols generally constitute the resting condition (Kim et al., 2002).

Nitric oxide (NO) is a free radical, and reacts with intracellular thiol, iron–sulfur clusters and heme targets present in many enzymes, receptors and ion channels, enabling NO to regulate many cellular processes (Stamler, 1994). While NO is a free radical, it possesses low reactivity with lipid membranes and has a limited effect on lipid peroxidation under physiological conditions (Beckman & Koppenol, 1996). Background levels of NO in muscle are in the low picomolar range (Roberts, Barnard, Jasman, & Balon, 1999), and are likely rapidly scavenged by myoglobin (Eich et al., 1996) and other cellular anti-oxidants (Wakulich & Tepperman, 1997). Nitric oxide itself may act as an antioxidant, but under conditions of oxidative stress, NO can propagate with other free radicals to form peroxynitrite (ONOO⁻), the degradation products of which are highly reactive with lipid membranes (Beckman, Beckman, Chen, Marshall, & Freeman, 1990).

Nitric oxide is a free radical that is constantly produced/released throughout the body by diverse tissues (Esch et al., 2002) including endothelium, brain and muscle tissue. The enzyme nitric oxide synthase (NOS) converts arginine and molecular oxygen to NO and citrulline in a reaction that requires NADPH, FAD, FMN and tetrahydrobiopterin as co-factors (Grozdanovic, 2001). The NOS family of enzymes contains three isoforms, nomenclature of the three isoforms follows the tissues they were initially isolated from: endothelial NOS (eNOS) (Janssens, Simouchi, Quertermous, Bloch, & Bloch, 1992; Lamas, Marsden, Li, Tempst, & Michel, 1992), macrophage or inducible NOS (iNOS) (Stuehr, Gross, Sakuma, Levi, & Nathan, 1989) and neuronal NOS (nNOS) (Wu, Williams, & McLoon, 1994). Nakane, Schmidt, Pollock, Forstermann, and Murad (1993) identified nNOS as the most prevalent isoform in skeletal muscle, and its expression in muscle was greater than in the brain. The three distinct isoforms of NOS that have been isolated are increasingly referred to as NOS-1 (nNOS), NOS-2 (iNOS) and NOS-3 (eNOS) in recognition of the wide tissue distribution of each isoform and this nomenclature will be used hereafter. Nitric oxide synthases are classified as constitutive (calcium activated) or inducible in their activity, but this has proved unreliable as a means of classification since each isoform may be regulated dynamically (Stamler & Meissner, 2001) and NOS-2 can be expressed constitutively (Guo et al., 1995; Park, Park, & Krishna, 1996). However, typically NOS-1 and NOS-3 activities are calcium dependant (Michel & Feron, 1997) and while NOS-2 activity is not influenced by calcium, it does contain a calmodulin binding domain (Bredt & Snyder, 1994). Nitric oxide has been demonstrated to play a crucial role in skeletal muscle, emerging as a model of nitric oxide function and redox-related signalling in biology (Stamler & Meissner, 2001).

Expression, activity and distribution of NOS in muscle vary between species and muscles. For example Kobzik, Reid, Bredt, and Stamler (1994) observed considerably higher content of NOS-1 in type II fibres than in type I rat skeletal muscle while others have observed NOS-1 in both fibre types in rat facial muscles (Tews et al., 1997) and in humans and primates (Grozdanovic, 2001). Planitzer, Miethke, and Baum (2001) reported that in rat skeletal muscle, fast-twitch oxidative fibres express the most NOS-1, relative to fast-glycolytic or slow-glycolytic fibres. All three NOS isoforms are expressed in skeletal muscle tissue (Stamler & Meissner, 2001), but vary in their sub-cellular and organelle distribution. NOS-1 in skeletal muscle is expressed in surface membranes (Nakane et al., 1993), where it is associated with membrane bound dystrophin complexes (Chang et al., 1996), sarcoplasmic reticulum (Xu, Huso, Dawson, Bredt, & Becker, 1999), neuromuscular junctions (Frandsen, Lopez-Figueroa, & Hellsten, 1996; Kobzik et al., 1994), myotendinous junctions and costameres (Chang et al., 1996).

Muscle NOS activity has been quantified by the conversion of ³H-L-arginine to ³H-L-citrulline and depending on species, resting skeletal muscle NOS activity...
generally varies between 2 and 25 pmol min\(^{-1}\) mg\(^{-1}\), averaging at about 10 pmol min\(^{-1}\) mg\(^{-1}\) (Stamler, 1994). Nitric oxide synthase activity in isolated skeletal muscles is increased during contraction by various electrical stimulation protocols (Kobzik et al., 1994; Reiser, Kline, & Vaghy, 1997; Stamler & Meissner, 2001) and exercise (Roberts et al., 1999). Increases in higher oxides of NO (NO\(_x\)) in human plasma have been correlated to both acute bouts of exercise and increases in physical fitness over time (Jungersen, Ambring, Wall, & Wennmalm, 1997). Inhibition of NOS activity with arginine analogues reduces muscle weight gain and sarcomere addition after atrophy in rats (Koh & Tidball, 1999).

NOS-1 is found in high concentrations in skeletal muscles, where its synthesis product NO is reported to affect skeletal muscles from deleterious effects of reactive oxygen species over the life span of an animal (Kaminski et al., 1997). Due to the close relationship between muscle contraction and NOS activity, it is likely that increases in muscle fibre NOS activity are associated with the contractile response to stress.

4. Types of pharmacological donors of NO and inhibitors of NOS

An understanding of the range of NO donors and NOS inhibitors assists in understanding the complexity of the system and the possible reasons for variation between studies. Pharmacological compounds are used to experimentally increase or decrease the effects of free NO in a biological system. The term “nitric oxide” is used to loosely describe the many redox states of NO. NO can be quenched by other free radicals (Szalai & Brudvig, 1996). Nitric oxide differs primarily from reactions with transition metal complexes and diatomic oxygen (Stamler, Singler, & Llosalzo, 1999), but NO can be quenched by other free radicals (Szlai & Brudvig, 1996). Nitric oxide differs from other signalling molecules as it does not propagate signals by ligand binding, but rather by covalent binding with redox sensitive biomolecules. The reactivity of NO with different biomolecules is characteristically different with the different redox states of NO and is a product of the surrounding redox milieu (Stamler, 1994).

NO is involved in stress physiology and stress-related diseases. Like stress, NO seems to be capable of exerting either strongly ameliorating (beneficial) or deleterious effects and counteracts norepinephrine activity and sympathetic responsiveness (Esch et al., 2002). NOS-1 appears to be involved in the neuroendocrine-immune response to stress, perhaps via glucocorticoid regulation (Bilbo, Hotchkiss, Chiavegatto, & Nelson, 2003) and is also active in the areas of the brain related to the stress reaction in response to restraint stress in rats (de Oliveira et al., 2000). In rats in which NOS inhibition is applied, through the use of the arginine analogue L-NAME (N\(^\circ\)-nitro-L-arginine methyl ester hydrochloride), the response to acute environmental stress was exaggerated (Kawa et al., 2002). Heat stress is associated with nitric oxide release (Sachidhanandam, Low, & Mookhala, 2002) and in humans working under extreme stress conditions, the severity of the stress symptoms was higher in workers with lower plasma NO concentration (Yeh, Leckman, Wan, Shah, & Lu, 2002).

The activity of NO synthase (NOS) in skeletal muscle is increased during exercise (Jungersen et al., 1997). Increases in higher oxides of NO (NO\(_x\)) in human plasma have been correlated to both acute bouts of exercise and increases in physical fitness over time (Jungersen et al., 1997). Due to the close relationship between muscle contraction and NOS activity, it is likely that increases in muscle fibre NOS activity are associated with the contractile response to stress.
5. Nitric oxide activity postmortem and meat tenderness

The use of NO to improve meat quality is not new as reduction of nitrates and nitrites to yield NO has traditionally been used for curing meat, increasing shelf life and improving colour (Cornforth, 1996; Walters & Taylor, 1964). Nitric oxide is known to react with superoxide to form peroxynitrite, which can initiate lipid oxidation and oxidation of oxymyoglobin to metmyoglobin post-slaughter, affecting the sensory quality and shelf life of meat (Ladikos & Lougovois, 1990). However, it is only recently that a link between NO synthesis and fresh meat tenderness has been defined, as first reported by Cook, Scott, and Devine (1998). In the study by Cook et al. (1998), injection of NO enhancers into hot-boned beef *longissimus lumborum* at 2 h post-slaughter resulted in more tender meat during ageing over 2–6 days and injection of NO inhibitors resulted in tougher meat over the same time period (Fig. 1). The enhancement of meat tenderness with the NO enhancers observed by Cook et al. (1998) occurred despite isoforms of NOS being sensitive to degradation by calpain (Laine & de Montellano, 1998; Walker, Pfeilschifter, Otten, & Kunz, 2001). Subsequently, Cottrell, Warner, and Dunshea (2002c) injected 1, 10 and 100 mM concentrations of NO enhancers and inhibitors into hot-boned beef *longissimus lumborum* at 2–3 h post-slaughter and found no effect on meat tenderness. There may be several reasons for the discrepancies between the two studies. When Cook et al. (1998) used injection of nitric oxide donors and NOS inhibitors into beef meat post-slaughter to demonstrate effects of NOS activity on meat tenderness, the injection occurred at 2 h post-slaughter, thus relying on availability of the necessary co-factors in pre-rigor meat. As they injected supraphysiological levels of the donors and inhibitors of NOS it is unlikely that the effects relate to NOS activity in postmortem meat. The lack of effect of injection of NOS inhibitors and NO donors on beef meat tenderness in the work of Cottrell et al. (2002c) was thought to be because the meat was close to rigor due to the use of high voltage electrical stimulation on the carcasses prior to muscle removal and thus the availability of co-factors for the NOS enzyme would have been limited.

Finally, one must be careful when making direct comparisons between studies using different NOS inhibitors and donors, as discussed in Section 4. Cook et al. (1998) used a cocktail of NOS inhibitors containing 1-NAME and 1-NOARG (N\textsubscript{o}-nitro-l-arginine) and a cocktail of NO donors containing SNAP (S-nitroso-N-acetylpenicillamin) and diethylenetriamine/NO adduct. Cottrell et al. (2002c) used SNP (sodium nitroprusside) as an NO donor and the same NOS inhibitors as those used by Cook et al. (1998).

Brannan and Decker (2002) investigated nitric oxide synthase activity post-slaughter and found that although chicken, turkey, pork diaphragm and trout muscle had NOS activity at 0 h post-slaughter, only the pork diaphragm retained NOS activity until 24 h post-slaughter and even then, it was in a system where ample substrate and co-factors were provided. They concluded that the ability of skeletal muscle to produce nitric oxide appears to be an event of relatively short duration as the co-factors that are required for the NOS reaction to generate NO (NADPH, FMN, FAD, tetrahydrobiopterin and oxygen) are most likely lost during postmortem storage. They also stated that future research on peroxynitrite-induced oxidation in muscle should focus on ante-mortem muscle biochemistry, handling during slaughter, and pre-rigor events in the muscle. This is the topic of the next section.

6. Nitric oxide activity in vivo and postmortem meat tenderness

Cottrell, Dunshea, McDonagb, and Warner (2002a, 2002b) were the first to show an effect of manipulation of NO levels in vivo on meat tenderness postmortem. They showed that infusion of a NOS inhibitor, 1-NAME, into lambs at 3 h pre-slaughter resulted in more tender meat in the *longissimus thoracis et lumborum* (LTL) muscle at 3 days post-slaughter and tougher meat in the *semimembranosus* (SM) at 1 and 3 days post-slaughter (Table 1). Ponnampalam, Warner, Dunshea, and Cottrell (2005) also used infusion of 1-NAME at 3 h pre-slaughter and found tougher meat in the SM at 1 but not 3 days post-slaughter (Table 1). Suster, Dunshea, Ponnampalam, Cottrell, and Warner (2005) (Table 1) infused 1-NAME at 3 and 24 h pre-slaughter and showed more tender meat in the SM and the LTL using Warner-Bratzler peak shear force and these differences were confirmed by myofibrillar fragmentation.
index values on the same muscles being significantly higher (indicates more tenderness) with NOS inhibition. The improvement in meat tenderness of the loin with NOS inhibition in all three studies was thought to be due to increased proteolytic activity, since NOS inhibition changed tenderness independently of sarcomere length, water holding capacity and pH. It is unclear why NOS inhibition conversely made the semimembranosus tougher. NOS inhibition using the same arginine analogues was observed by Cook et al. (1998) to increase toughness in beef loin. The original postulate of the work by Cottrell et al. (2002a, 2002b) was that pre-slaughter NOS inhibition would mimic this effect found by Cook et al. (1998). However, this was not supported in the experiment by Cottrell et al. (2002a, 2002b) since NOS inhibition improved tenderness in the LTL, although the SM was tougher with NOS inhibition. The differential effects of arginine analogues between the experiments indicate that injection into meat and the pre-slaughter infusion of arginine analogues affect meat quality via different mechanisms.

It is unclear why the effects of NOS inhibition using L-NAME on lamb meat tenderness varied between muscles and also between experiments and this probably indicates our lack of understanding of the system. Table 1 also shows that the change in muscle tenderness in response to L-NAME can depend on exercise levels in the animal, provision of NO donors (arginine) and also the time of infusion. Planitzer et al. (2001) reported differences in NOS-1 distribution between type IIa, IIb and type 1 fibre types and muscles but as the SM and LTL muscles are generally considered to have similar distribution of the fibre types (Totland & Kryvi, 1991), this is unlikely to explain the difference. It is more likely due to differences in muscle use pre-slaughter as the SM is considered a muscle which is actively contracting during walking and running whereas the LTL is a postural muscle (Totland & Kryvi, 1991). Cottrell, Warner, McDonagh, and Dunshea (2004) reported that the effect of NOS activity inhibition with L-NAME on skeletal muscle metabolism was dependent on the degree of NOS stimulation through exercise or the lack of exercise. The semimembranosus muscle has higher levels of calpastatin activity in pig meat (Melody et al., 2004) and in sheep meat (Duckett, Snowder, & Cockett, 2000) thus it is possible that NOS regulation of calpain activity is dependent on the concentration and activity of the inhibitor.

The total collagen concentration of the semimembranosus muscle is much higher then that of the longissimus in beef muscle (7.68 vs. 4.52 mg/g, respectively, Rhee, Wheeler, Shackelford, & Kooohmarae, 2004; 0.58% vs. 0.41%, respectively, Eikelenboom, Barnier, Hoving-Bolink, Smulders, & Culioli, 1998) and the semimembranosus also has a lower heat-soluble collagen content then the longissimus in achilles hung beef muscle (10.8% vs. 13.2%, respectively, Eikelenboom et al., 1998). A recent in vitro experiment has demonstrated that extracellular matrix turnover is tightly regulated and both NO and matrix metalloproteinsases (MMP) play a pivotal role (Lopez-Rivera et al., 2005). Application of NO to endothelial cells disrupted a complex between MMP-13 and caveolin-1, leading to collagen breakdown (Lopez-Rivera et al., 2005), implicating involvement of NO in collagen turnover. Thus the muscle difference in the response of Warner-Bratzler peak

### Table 1

Effects of nitric oxide synthase inhibition using L-NAME infusion (control, saline, 0 mg of L-NAME/kg vs. 30 mg of L-NAME/kg) into the jugular via an indwelling catheter, time of infusion (3 h vs. 24 h pre-slaughter) and exercise (− Exercise vs. + Exercise) or arginine infusion (−Arg vs. + Arg) on Warner-Bratzler peak shear force (WB, kg) in lamb longissimus thoracis et lumborum (LTL) and semimembranosus (SM) in three separate experiments.

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>L-NAME</th>
<th>SED</th>
<th>P value</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>WB-LTL</td>
<td>9.79</td>
<td>8.58</td>
<td>0.372</td>
<td>0.002</td>
<td>Cottrell et al. (2002b)</td>
</tr>
<tr>
<td></td>
<td>− Exercise</td>
<td>+ Exercise</td>
<td>− Exercise</td>
<td>+ Exercise</td>
<td></td>
</tr>
<tr>
<td>WB-SM</td>
<td>6.15</td>
<td>7.05</td>
<td>7.8</td>
<td>7.35</td>
<td>0.044</td>
</tr>
<tr>
<td>WB-LTL</td>
<td>5.92</td>
<td>5.72</td>
<td>0.44</td>
<td>0.28-0.86</td>
<td>Ponnampalam et al. (2005)</td>
</tr>
<tr>
<td>WB-SM</td>
<td>4.38</td>
<td>4.73</td>
<td>0.22</td>
<td>0.03</td>
<td></td>
</tr>
<tr>
<td></td>
<td>− Argb</td>
<td>+Argb</td>
<td>− Arg</td>
<td>+Arg</td>
<td></td>
</tr>
<tr>
<td>WB-SM</td>
<td>4.56</td>
<td>4.2</td>
<td>4.57</td>
<td>4.89</td>
<td>0.22</td>
</tr>
<tr>
<td>WB-LTL</td>
<td>4.13</td>
<td>3.71</td>
<td>0.134</td>
<td>0.002</td>
<td>Suster et al. (2005)</td>
</tr>
<tr>
<td>WB-SM</td>
<td>3.72</td>
<td>3.51</td>
<td>0.013</td>
<td>0.047</td>
<td></td>
</tr>
<tr>
<td></td>
<td>3 h</td>
<td>24 h b</td>
<td>24 h</td>
<td></td>
<td></td>
</tr>
<tr>
<td>WB-LTL</td>
<td>3.97</td>
<td>4.24</td>
<td>3.81</td>
<td>3.61</td>
<td>0.170</td>
</tr>
</tbody>
</table>

* − Arg = control, 30 mL 0.9% NaCl; +Arg = 500 mg/kg body weight L-arginine in 0.9% saline.

* 3 h = infusion at 3 h pre-slaughter, 24 h = infusion at 24 h pre-slaughter.
shear force to NOS inhibition may be due to differing effects of NO production on collagen breakdown.

The discrepancies between studies and muscles highlight the need for further research in the area. Since inhibition of NOS with L-NAME was observed to improve light the need for further research in the area. Since inhibition of NOS by NOS-1 increases SR Ca\textsuperscript{2+} release by nitrotyrosylation of a cysteine residue in the RYR protein. Both SERCA and RyR are rich in redox sensitive cysteine residues, which are sensitive to nitrosylation and oxidation (Xu, Zweier, & Becker, 1997). Cysteine oxidation by NO or reactive oxygen intermediates (ROIs) influences the function of SERCA and RyR (Stamler et al., 2001; Xu et al., 1997). In particular, Sun, Xin, Eu, Stamler, and Meissner (2001) demonstrated that separate cysteine residues within the RyR were adapted to mediate nitrosative and oxidative stimuli. Hart and Duhlunty (2000) observed that the cardiac RyR activity was increased by low concentrations and inhibited by high concentrations of a NO donor. This led to the hypothesis that the effects of NO on contraction are mediated by its concentration. Furthermore, O\textsubscript{2} concentration dynamically controls the redox state or 6–8 thiols per RyR subunit thereby regulating S-nitrosylation of the channel thiol by nitric oxide (Eu, Sun, Xu, Stamler, & Meissner, 2000) and calcium binding to calmodulin is required to unmask the critical thiol for S-nitrosylation (Stamler et al., 2001).

NO is also known to inhibit the sarcoplasmic/endoplasmic reticulum Ca-ATPase (SERCA) resulting in higher cytosolic calcium (Hare, 2003). See Fig. 2 for a proposal of how NO and L-NAME may influence protein degradation through its effects on Ca homeostasis.

7.2. Calpain and NO

Oxidative damage to proteins is an adjunct of aerobic metabolism and removal of oxidatively damaged proteins by proteolytic enzymes is integral to normal cell cycling (Mehlhase & Grune, 2002). However, the proteolytic enzymes themselves are in turn regulated

7.1. Ca homeostasis and NO

NO has been demonstrated to influence muscle contraction in numerous species, partly due to the regulatory effect of NO on SERCA and RyR (Stamler & Meissner, 2001). Among the various activators and inhibitors of RyRs, the oxidation and reduction of RyRs has shown to be one of the most potent mechanisms that can reversibly open and close the channel (Cheong, Tumbev, Abramson, Salama, & Stoyanovsky, 2005). Both excitation and inhibition of sarcoplasmic/endoplasmic reticulum ATPase (SERCA) and ryanodine receptor (RyR) by NO have been observed, depending on the concentration and redox chemistry of NO, concentrations of NO scavenging molecules and methodology used (Stamler, Lamas, & Fang, 2001). NOS-1 is in close proximity to the RYR and release of NO by NOS-1 increases SR Ca\textsuperscript{2+} release by nitrotyrosylation of a cysteine residue in the RYR protein. Both
by reactive nitrogen and oxygen species. While proteasome and cathepsin activities are sensitive to oxidation (Strack, Waxman, & Fagan, 1996), it is the interactions with calpain that may prove most important for meat quality. This can occur indirectly as oxidative stress can increase cellular calcium and increase calpain activity resulting in cytoskeletal damage (Mehlhase & Grune, 2002). As with other physiological systems, the effects of oxidation on calpain activity are concentration dependent. (Pronzata et al., 1993) observed that low levels of carbon tetrachloride (CCl₄) induced injury stimulated calpain activity, while high doses of CCl₄ inhibited calpain activity.

Alternatively, inhibition of calpain can occur via direct interactions. Calpain contains cysteine (Sorimachi et al., 1993), making it susceptible to S-nitrosylation by NO. Reversible inhibition of calpain by NO donors has been observed in separate studies (Koh & Tidball, 2000; Michetti et al., 1995). However, in what may constitute a cell signalling pathway, NOS isoforms are sensitive to degradation by proteasome (Musial & Eissa, 2001) and calpain (Walker et al., 2001).

NOS-1 has a PDZ region which interacts with other proteins including with caveolin-3, where it appears to comprise a component of the dystrophin complex (Venema, Ju, Zou, & Venema, 1997). The calpain and ubiquitin–proteasome pathways are the major proteolytic systems responsible for the regulated degradation of NOS-1 and this is influenced by diverse agents including glucocorticoids, caveolin-1, heat shock proteins (hsp90) and certain NOS inhibitors (Kone et al., 2003).

Endogenous NO is involved in inducing hypertrophy of skeletal muscle fibres by decreasing protein degradation and increasing protein synthesis (Koh & Tidball, 2000). NO regulates calpain-initiated cytoskeletal degradation and Zhang, Kraus, and Truskey (2004) showed that exposure of myotubes in culture to L-NAME caused increased proteolysis, measured by the change in elasticity modulus, which was reversed by addition of a calpain inhibitor. NO also plays an important role in preventing calpain-activated proteolysis of the cytoskeleton, particularly talin and vinculin, as demonstrated by stabilisation of talin by an NO donor that prevents calpain-activated-proteolysis of the cytoskeleton and reduced degradation and increased synthesis of vinculin in the presence of NO (Zhang et al., 2004). To a lesser extent, the synthesis of the intermediate filament desmin was greater in L-NAME treated samples (Zhang et al., 2004). As desmin has been linked to the development of meat tenderness during ageing (Taylor, Geesink, Thompson, Kooimaraie, & Goll, 1995; Whipple et al., 1990), this may contribute to the observed effects of NOS inhibition on meat tenderness. Wheeler, Shackelford, and Kooimaraie (2002) showed that 64% of the variation in beef longissimus tenderness could be explained by variations in desmin degradation. Desmin increases stiffness of the diaphragm muscle in a transverse as well as in a longitudinal direction (Boriek et al., 2001). It may be that the most important component of meat tenderness is derived from transverse stress on the meat during chewing as well as longitudinal stress.

NO donors have been shown to inhibit calpains (Forsythe & Fefus, 2003; Michetti et al., 1995) and cathepsins (Ascenzi et al., 2001). Michetti et al. (1995) specifically reported that the NO donor sodium nitroprusside (SNP) inhibits m-calpain at neutral pH and µ-calpain at acidic pHs. See Fig. 2 for a proposal of how NO and L-NAME may influence protein degradation directly through affecting calpain.

8. Oxidation environments pre- and post-slaughter and quality

Feeding of Vitamin E to cattle increased tissue z-tocopherol, which protects membrane lipids and myoglobin from oxidation (Liu, Lanari, & Schaefer, 1995). Subsequently, this delayed onset of discoloration in fresh, ground and frozen beef (see Liu et al., 1995 for a review). Consumer assessed tenderness has been found to increase with on-farm Vitamin E supplementation in pigs and rabbits (Dal Bosco, Castellini, Bianchi, & Muggnai, 2004; Kerth et al., 2001), decrease in tenderness in beef (Robbins et al., 2003) and show no change in pigs and beef in other studies (Arnold et al., 1992; Waylan et al., 2002). Tenderness measured by Warner–Bratzler peak shear force (WBPSF) has been shown to tend to increase with Vitamin E supplementation in beef at 15 days post-slaughter (Rowe et al., 2004b), a tendency to decrease in pigs (Waylan et al., 2002) and show no change in two studies in beef (Arnold et al., 1992; Robbins et al., 2003). Furthermore, Realini, Duckett, Brito, Dalla Rizza, and De Mattos (2004) reported that the longissimus muscle from pasture fed cattle had a higher z-tocopherol concentration and was also more tender at both 7 and 14 days postmortem compared to the longissimus from concentrate-fed cattle. Overall, there would appear to be a tendency for Vitamin E fed cattle to produce more tender meat but this was not consistent across all studies. Oxidation induced by irradiation of beef loin early postmortem increases Warner–Bratzler tenderness values during ageing and this was confirmed to be due to decreased proteolysis measured by appearance of protein degradation products on Western blots and by decreased µ-calpain activity (Rowe et al., 2004b). Increasing protein oxidation induced by irradiation has been shown to be associated with increased shear force values at later times postmortem (Rowe, Maddock, Lonergan, & Huff-Lonergan, 2004a). Vitamin E supplementation reduces the oxidation of sarcoplasmic proteins although no effects on shear force
were observed (Rowe et al., 2004b). The authors conclude that oxidative processes in early postmortem meat may hinder tenderisation by interfering with proteolytic processes.

Oxidation of lipid, myoglobin and other proteins is also important during storage of meat due to development of off-odours and flavours, discoloration to unacceptable brown metmyoglobin and problems with tenderness. Meat is traditionally aged in a vacuum bag that excludes oxygen but modern plants utilising centralised packaging have moved towards using modified atmosphere packaging. As modified atmosphere packaging usually includes a small amount of oxygen, oxidation processes in the meat proceed more rapidly then under vacuum packing as evidenced by the increased discolouration (Schluter et al., 1994). Several studies have shown that over the same time period, vacuum packaged beef meat exhibits superior tenderisation relative to meat packed under modified atmosphere with varying concentrations of O2 (Fu, Molins, & Sebranek, 1992; Griffin et al., 1982). Furthermore, Sorheim, Wahlgren, Nilsen, and Lea (2004) showed that “LD steaks displayed in the high O2 atmosphere had reduced development of sensory and instrumental tenderness”.

A number of studies investigating modified atmosphere packaging routinely measure lipid oxidation (by TBARS method), pigment oxidation [by measuring surface colour (CIE or Hunter L*, a*, b*, hue, saturation)] and consumer assessments of discoloration, microbial spoilage organisms and off-odours but the limited reports of negative effects of modified atmosphere packaging on tenderisation during ageing warrant further research. The involvement of calpains in meat tenderisation has been demonstrated in numerous experiments and according to (Koohmaraie, 1994), calpain is the principal proteolytic enzyme for tenderising meat. It is important to quantify the prevalence of protease oxidation during postmortem storage in MAP if it is limiting the tenderisation process.

9. Effects of nitric oxide on postmortem metabolism

The importance of muscle metabolism to meat quality is highlighted by the development of dark cutting (DC) and dark firm dry (DFD) meat, which occurs due to reduced conversion of glycogen to lactate post-slaughter (Lawrie, 1958). Dark-cutting has a high ultimate pH (pHu) and a curvilinear relationship between pHu and tenderness has been observed, where meat of an “intermediate” pHu of approximately 6 is less tender than meat with higher or lower pHu values (Bouton, Harris, & Shorthose, 1971; Watanabe, Daly, & Devine, 1996). As observed by Watanabe et al. (1996), the increased toughness associated with intermediate pHu meat is removed by ageing. Depletion of muscle glycogen can occur by endocrine responses to stressful stimuli including adrenaline release or increased physical activity (Lacourt & Tarrant, 1985). Nitric oxide (NO) has emerged as an important regulator of skeletal muscle homeostasis, where it interacts with many physiological pathways influencing meat quality. Nitric oxide may prove a useful agent for improving meat quality as it influences many systems important to meat quality. This potentially includes protecting against “dark cutting”, which is normally a result of stress induced glycogen depletion pre-slaughter (Lawrie, 1958). Glycogen depletion may be due to the ability of NO to influence muscle glucose uptake (Balon, Jasman, & Young, 1999; Etgen, Fryburg, & Gibbs, 1997), glycogenolysis (Borgs et al., 1996; Jaffrey, Erdjument-Bromage, Ferris, Tempst, & Snyder, 2001) and glycolysis (Mohr, Stamler, & Brune, 1996).

The inhibitory effect of l-NAME on exercise-induced hyperglycaemia indicates that NO influences glucose homeostasis during exercise. Inhibition of basal and stimulated glycogen synthesis by the NOS inhibitor, SNP, in rat soleus muscles has been observed (Young & Leighton, 1998b) and inhibited insulin-stimulated, but not basal glycogen synthesis (Young, Radda, & Leighton, 1997). While NO has also been demonstrated to play an inhibitory role in hepatic glycogenolysis (Borgs et al., 1996) as well as in glycogen synthesis (Young & Leighton, 1998a), little is known about the effects of NO on muscle glycogenolysis post-slaughter. Cottrell et al. (2002a, 2002b) infused l-NAME into lambs at 3 h pre-slaughter and reported post-slaughter reductions in muscle glycogen concentration and increased lactate production in both the LTL and SM muscles as well as an average reduction in LTL pH by 0.06 pH units. They postulated that it was unlikely that the small differences in pH and glycogenolysis were unlikely to have been the factor determining the increase in tenderness of the LTL and the increased toughness in the SM with l-NAME infusion in the same study (Cottrell et al., 2002a, 2002b). Other experiments using pharmacological NO donors have observed increased lactate production in skeletal muscle (Young & Leighton, 1998b; Young et al., 1997), in contrast to the effect of l-NAME in the experiment by Cottrell et al. (2002a, 2002b). It is most likely that any interaction between NO release and muscle metabolism around slaughter would only have an effect on meat quality through effects on muscle glycogen concentration and therefore the dark-cutting condition.

10. Conclusions and future research

NOS activity pre-slaughter and possibly postmortem appears to have an effect on meat tenderisation. The effect of the inhibition of NOS in vivo on meat tenderness
varies with muscle and is also likely to vary with species, temperament, stress and exercise. The prevalence of the different various NOS isoforms in various muscles in high value cuts needs to be investigated. The conditions under which NO are released in skeletal muscle between farm and slaughter warrants investigation as well as the importance of the timing of the release relative to slaughter. The impact of postmortem oxidative conditions on protease activity including calpains, cathepsins and other proteolytic enzymes is of interest in the production of consistently tender meat. It may be possible to enhance the antioxidant status of the animal to ensure that both tenderness and shelf life in MAP are maximised.

In conclusion, we propose that meat tenderisation postmortem is influenced by skeletal muscle’s release of NO pre-slaughter and the oxidation of proteases postmortem. This proposal is compatible with the existing tenderness model and will hopefully assist in increasing the accuracy of prediction of meat tenderness.

References


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