EFFECTS OF POSTMORTEM GLYCOLYSIS ON THE QUALITY OF HOT-DEBONED BOVINE MUSCLE

H. L. $BRUCE^{\perp}$ and R. O. $BALL^{1,2}$

¹Department of Animal and Poultry Science, University of Guelph, Guelph, Ontario, Canada N1G 2W1; and ²Department of Nutritional Sciences, Faculty of Medicine, University of Toronto, Toronto, Ontario, Canada M5S 1A8. Received 27 June 1989, accepted 5 Dec. 1989.

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Meat tenderness may be improved by accelerating muscle metabolism or by damaging muscle structure and increasing the solubility of muscle proteins. Pre- and postrigor protein and collagen solubilities were measured in semitendinosus muscles, removed pre-rigor from 24 Charolais crossbred steer carcasses, that were either unstimulated or electrically stimulated (115 V, 0.25 amp, 60 Hz) within 1 h postexsanguination to accelerate muscle metabolism. Temperature, pH, Hunterlab color reflectance, sarcomere length and lactate concentration were measured during ageing. Shear force was measured on aged (7 d) muscle only. Low voltage electrical stimulation increased glycolytic rate as indicated by significantly (P < 0.05) lower pH and higher L-lactate concentrations of stimulated muscles as compared to control muscles. Total and sarcoplasmic protein solubilities decreased due to ageing, and myofibrillar protein solubility increased; however, collagen solubilities were unchanged. Low voltage electrical stimulation did not affect color reflectance, sarcomere length or shear force, indicating that an increased rate of glycolysis alone was not sufficient to effect increases in meat tenderness.

Key words: Beef, electrical stimulation, meat quality, muscle

[Effets de la glycolyse post-mortem sur la qualité des muscles de bovins désossés à chaud.]

Titre abrégé: Glycolyse post-mortem des muscles et qualité de boeuf.

On peut accroître la tendreté de la viande en accélérant le métabolisme des muscles ou en endommageant la structure musculaire et en augmentant la solubilité des protéines musculaires. La solubilité des protéines et du collagène pré-rigor et post-rigor a été mesurée dans les muscles demitendineux, prélevés avant l'établissement de l'état de rigidité, sur 24 carcasses de bouvillons Charolais croisés non stimulés ou stimulés électriquement (115 v, 0,25 A, 60 Hz) dans l'heure suivant l'exsanguination pour accélérer le métabolisme musculaire. La température, le pH, la réflectance de la couleur Hunterlab, la longueur des sarcomères et la concentration en lactate ont été mesurés durant le processus de vieillissement. La résistance au cisaillement n'a été mesurée que sur les muscles vieillis (7 jours). La stimulation électrique à faible voltage a augmenté la glycolyse, comme en témoigne la baisse significative (P < 0.05) du pH et la hausse de la concentration en L-lactate des muscles stimulés par rapport aux témoins. La solubilité des protéines totales et sarcoplasmiques a dimunué sous l'effet du vieillissement, alors que celle des protéines des myofibrilles a augmenté; la solubilité du collagène est toutefois demeurée inchangée. La stimulation électrique à faible voltage n'a pas eu d'effet sur la réflectance de la couleur, la longueur des sarcomères ou la force de cisaillement, ce qui indique que l'accroissement de la glycolyse n'est pas suffisant à lui seul pour accroître la tendreté de la viande.

Mots clés: Boeuf, stimulation électrique, qualité de la viande, muscle

Electrical stimulation has been suggested to improve meat tenderness by accelerating postmortem muscle metabolism (Davey et al. 1976). Rapid decline of pH early postmortem has been suggested to be desirable because the combination of low pH and high temperature appears conducive to proteolytic activity and increased myofibrillar protein solubility (Yates et al. 1983). However, more recent research indicated that low pH early postmortem decreased meat tenderness (Marsh et al. 1981; Unruh et al. 1986), and that electrical stimulation may increase meat tenderness by inflicting structural damage to muscle through induction of tetanic contractions (Takahashi et al. 1984). Thus, the literature implies that electrical stimulation of beef carcasses may be of little consequence unless there is disruption of the sarcomere series. An increase in tenderness therefore should be greatest in muscles that are restrained in situ during electrical stimulation, because the anatomical restraint during contraction would produce more fiber disruption. Conversely, muscle that is not restrained during electrical stimulation should have little muscle fiber disruption and consequently no increase in tenderness despite changes in postmortem muscle metabolism.

Electrical stimulation of hot-deboned muscle with a low voltage, high frequency (60 Hz) current accelerates muscle metabolism markedly (Smulders et al. 1986). A high-frequency (60 Hz) current inflicts damage to the muscle fibers because the rapid pulses of current do not allow the muscle to relax between contractions; hence, provoking a tetanic response (Takahashi et al. 1984). However, without anatomical restraint applied to the muscle from tendon sheath and bone attachments, the tetanic response should inflict less muscle fiber disruption while still providing a concomitant acceleration of anaerobic glycolysis, thus removing the aspect of damage from the effect of electrical stimulation. Because the meat-packing industry is progressively reducing the time that beef remains at the abattoir, investigation of how electrical stimulation may improve tenderness of hot-deboned muscles would be of industrial interest. Hot-deboned muscles, or muscles excised prior to rigor and cooling, are particularly susceptible to cold-shortening and therefore increasing the metabolic rate before cooling may prevent cold-shortening (Carse 1973; Babiker and Lawrie 1983). Electrical stimulation of hot-deboned muscle with high voltages following excision was addressed by Jeremiah et al. (1985); however, how hot-deboned muscle responds to low voltage electrical stimulation and moderate cooling upon excision does not appear to have been studied.

Therefore, an investigation of the effect of low voltage, high frequency (60 Hz) electrical stimulation on hot-deboned muscle was designed, with excision of the muscle thus removing the effect of muscle fiber disruption due to anatomical restraint. The main objective of this experiment was to isolate the effects of accelerated postmortem muscle metabolism from the effects of fiber disruption on meat quality.

MATERIALS AND METHODS

Experimental Design

Twenty-four Charolais steers from the Elora Beef herd at the University of Guelph, which had been finished to a Canada Grade A1 backfat of between 4 and 9 mm on a corn-corn silage diet and averaged 606 kg (± 9.2 kg standard error of mean) at 16 mo of age, were fasted overnight and slaughtered conventionally in the University abattoir. The animals entered the abattoir at random choice of the butcher; therefore, animals were allotted alternately to the treatment group (electrical stimulation) or to the control group (no electrical stimulation), beginning with the former. The leg used to suspend the carcass during exsanguination was noted, and the semitendinosus was removed from the opposite side, with excision and removal of the muscle taking place approximately 1 h postmortem (52.9 min SEM 4.52). This was done to reduce any effect of restraint on the muscle structure during exsanguination. The semitendinosus muscle was trimmed of all exterior fat and the epimysium was left intact. The semitendinosus muscle was selected because of its easily accessible location, its cylindrical shape and the longitudinal direction of its fibers.

Postmortem Muscle Metabolism

After weighing the muscle, internal muscle temperature was measured with a Taylor thermometer with a metal probe (Sybron Corporation, Arden, North Carolina) and the pH was measured using a pH meter with glass probe (Metrohm Herisau, Switzerland). The probe was inserted into a 5-cm scalpel cut that was made parallel to the muscle fibers. Approximately 30 g of the freshly excised muscle were removed with a scalpel, trimmed of epimysium and fat, frozen in liquid nitrogen, placed in a plastic bag and submerged in ice until the sample was vacuum-packaged and stored at -30°C for later analysis for prerigor collagen and protein solubility and lactic acid content.

Muscles designated to the treatment group received five 20-s pulses, resulting in 100 s of stimulation from a 115 V, 0.25 amp, 60 Hz alternating current (Jarvis BV-80 electrical stimulation unit, Jarvis, Middleton, Connecticut) and were stimulated approximately 5-min postexcision.

Following removal of the sample for drip loss, both stimulated and control muscles were vacuum-packaged and placed in a cooler for 4 h at 12° C, 19 h at 7° C, and 6 d at 2° C. The muscles were unpackaged for measurement of temperature and pH and removal of approximately 10 g of muscle for lactic acid measurement at 2, 4, 6 and 8 h post-stimulation and then repackaged. All muscles were frozen at -10° C following removal of color samples at 7 d of ageing.

L-lactic acid was extracted from the lyophilized meat samples according to the perchloric acid method provided in the L-lactic acid kit instructions and L-lactic acid analysis was completed with a reagent kit (Boehringer Mannheim, Dorval, Quebec). L-lactate (Sigma Chemical Company, St. Louis, Missouri) was used to construct a standard curve and determine lactate recovery from the perchloric acid procedure.

Drip Loss

Approximately 100 g of muscle with epimysium intact were sliced from both control and stimulated muscles at the insertion end of the muscle for drip loss according to Honikel (1987). The drip loss sample was suspended in a sealed bag at atmospheric pressure and placed in the same cooler as the muscles for 7 d, dried with an absorbent towel and reweighed.

Sarcomere Length

Sarcomere length was measured after 48 h of ageing with a phase-contrast microscope fitted with

a micrometer as described by Salm et al. (1983). Briefly, approximately 0.5 g of muscle was removed and homogenized in 30 ml of 0.08 M KC1 for 15 s in a 50-mL stainless steel Waring blender cup (Waring Commercial, New Hartford, Connecticut) at full speed. Myofibrillar segments containing at least six sarcomeres were measured on fifty randomly selected myofibrils from each sample and the average sarcomere length calculated.

Shear Force Analysis

Semitendinosus steaks, cut to thickness of 2.2 cm while frozen from the insertion end of the muscle, were thawed at 2°C overnight and roasted uncovered at 165°C until an internal temperature of 70°C was attained (Salm et al. 1983). The steaks were allowed to cool to room temperature for 4 h. Five cores per steak, each core 2 cm in diameter, were sheared perpendicular to the fiber direction with a Warner-Bratzler shear force machine, single blade, and the peak force was recorded.

Color Determination

Hunterlab color reflectance (Spectrogard Colour System, Pacific Scientific, Silver Spring, Maryland) was measured after 30 min of bloom time at room temperature from 2.2-cm-thick slices taken from the insertion end of the semitendinosus muscle at 48 h and 7 d postmortem.

Collagen and Protein Solubility Analyses

Sample preparation for collagen solubility analysis was performed according to Hill (1966), with hydroxyproline of the samples measured using the colorimetric method of Bergman and Loxley (1963). Hydroxyproline was converted to collagen using 7.25 for insoluble and 7.52 for soluble collagen (Cross et al. 1973). L-4-hydroxyproline (Sigma Chemical Company, St. Louis, Missouri) was used for a standard curve.

Total, myofibrillar and sarcoplasmic proteins were extracted using the method of Link et al. (1970), with the protein measured using the biuret method of Layne (1957). A standard curve was constructed with bovine serum albumin (Boehringer Mannheim, Dorval, Quebec). Total protein was determined by micro-Kjeldahl procedures (Association of Official Analytical Chemists 1975).

Statistical Analysis

Data were analyzed with Statistical Analysis System (Statistical Analysis System Institute, Inc.

1982) using models and statistical tests appropriate for each data set. Data involving comparisons between control and stimulated muscles (collagen and proteins solubilities, sarcomere length, color reflectance and shear force) were analyzed using t-tests (least square means) and analysis of variance. Data measured from repeated measurements (pH and temperature) were tested for differences between treatments within time. Data from two times (prerigor vs. postrigor) and treatments (unstimulated and stimulated) were compared using a 2 × 2 factorial design, with data blocked on treatment and time, and tested for treatment, time and treatment by time interaction with analysis of variance and least square means comparisons. Significant interactions were tested with Student-Newman-Keuls multiple range test to determine differences among means and t-tests to determine which main effect had the greatest influence. Analysis of covariance using prerigor myofibrillar protein solubility as covariate was performed on the postrigor myofibrillar protein solubility data to decrease variability due to a significant pretreatment difference in myofibrillar protein solubility between the stimulated and control muscles. Pearson correlations were also performed to correlate prerigor and postrigor collagen and protein solubilities, as well as to correlate meat quality measurements to shear force.

For drip loss data and 24-h color reflectance, *n* for both groups were reduced because these measurements were added following the beginning of the sampling. The *n* of the control group for the prerigor collagen and protein measurements was reduced by one because a prerigor sample was lost. In statistical sets with missing data, the General Linear Models procedure was used (Statistical Analysis System Institute, Inc. 1982).

RESULTS AND DISCUSSION

Growth and Carcass Characteristics

There were no significant differences between the control and stimulated animals for growth rate, excised hot muscle weight, hot carcass weight, loin eye fat cover, protein or fat content (Table 1). No differences between the treatments for these measurements indicated that there were unlikely to be differences in experimental effects due to muscle size or surface area.

Postmortem Metabolism

There was no significant difference between the control and stimulated muscle groups for

Table 1. Carcass and muscle characteristics of aged, excised semitendinosus: control vs. stimulated

	Control	Stimulated	SEM†
Liveweight (kg)‡	602	610	13
Carcass weight (kg)‡	360	355	6
Subcutaneous fat			
(mm)‡	7.40	6.50	0.70
Excised muscle weight			
(kg)‡	2.50	2.56	0.15
Drip loss (g kg ⁻¹)§	28.6	32.6	6.0
Sarcomere length			
(μm)‡	1.90	1.89	0.03
Peak shear force			
(kg)‡¶	8.04	7.88	0.64
Protein (g kg ⁻¹)‡	831.0	813.0	20.6
Fat (g kg-1)‡	118.8	130.3	14.8

†SEM = standard error of the mean.

 $\ddagger n = 12$ for control and stimulated groups.

 $\S{n} = 9$ for control, n = 8 for stimulated muscles.

Peak force expressed in kg/2.0 cm core.

Dry tissue basis.

temperature decline postexcision (Fig. 1). Stimulated muscles had significantly lower (P < 0.05) pH measurements 2, 4, 6 and 8 h poststimulation than the control muscles (Fig. 2). The significant increase in lactic acid in the stimulated muscles (Fig. 3), which was correlated to pH (r = -0.99, P < 0.01), indicated that the pH decline was indicative of accelerated glycolysis in the stimulated muscles. The increased rate of glycolysis may have also been accompanied by an increase in the temperature of the muscles had the muscles been insulated; however, the large surface area of the excised muscle prevented heat conservation and the cooling rate did not differ from that of the controls. The removal of any temperature differential was deliberate and necessary because temperature affects the rate of pH decline (Busch et al. 1967) and this would have confounded interpretation of the results.

Muscle Characteristics

The sarcomere lengths of the control and stimulated muscles (Table 1) indicated that the vacuum-packaged muscles did not cold shorten during cooling because the mean value was similar to the $1.98 \pm 0.08 \, \mu \text{m}$ of unrestrained, uncold-shortened semitendinosus as measured by Cross et al. (1981).

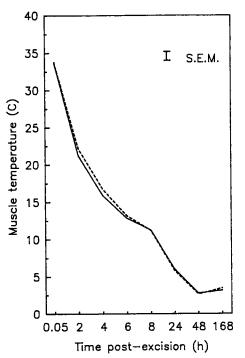


Fig. 1. Temperature of semitendinosus muscle at various intervals (h) postexcision: control vs. stimulated. SEM, standard error of mean. —, control; ---, stimulated.

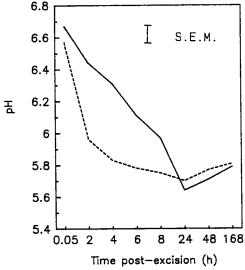


Fig. 2. Semitendinosus muscle pH at various intervals (h) postexcision: control vs. stimulated. SEM, standard error of mean. —, control; ---, stimulated.

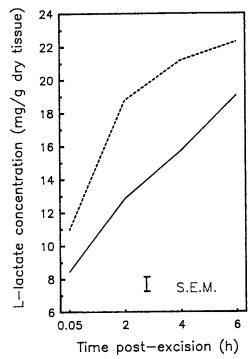


Fig. 3. L-lactate concentration of semitendinosus muscles at various intervals (h) postexcision: control vs. stimulated. SEM, standard error of mean.

—, control; ---, stimulated.

Electrical stimulation did not affect the sarcomere length (Table 1). Low voltage electrical stimulation of carcasses has been shown to reduce sarcomere shortening (Eikelenboom et al. 1985); however, this was in muscle aged in the carcass. Bouton et al. (1980) found that sarcomeres in electrically stimulated muscles were longer than control muscles earlier postmortem (1 and 2 h postslaughter), but not by 22 h postslaughter. Reduced shortening of the sarcomere may increase filament surface area and the accessibility of proteolytic enzymes to muscle proteins and thereby increase tenderness.

Low voltage electrical stimulation of beef carcasses has been found to increase drip loss (Eikelenboom et al. 1985); however, this was not so in this experiment (Table 1). The increased exudation of water from meat and the concomitant increase in brightness found in Eikelenboom et al. (1985) may be related

to the denaturation of sarcoplasmic and myofibrillar proteins (Scopes 1970) produced by electrical stimulation.

There was no significant difference between the control and stimulated muscles for peak shear force values of the cooked semitendinosus (Table 1) despite the acceleration of muscle metabolism, as indicated by the significant increase in the rate of pH decline and lactate content of the muscles (Figs. 2 and 3). This observation supports the hypothesis of various researchers (Savell et al. 1978; Marsh et al. 1981; Takahashi et al. 1987) that electrical stimulation increases tenderness not through promoting acidic conditions but through myofibrillar disruption. High frequency (60 Hz) electrical currents have been found to elicit myofibrillar damage whereas low frequency (2 Hz) currents have not (Marsh et al. 1981; Takahashi et al. 1987). Presumably, the low frequency (2 Hz) current does not allow the muscle to elapse into tetanic contractions, thereby preventing myofibrillar damage while still increasing the rate of pH decline. The current research supports this hypothesis because excision prior to electrical stimulation may have decreased the myofibrillar damage by allowing the muscle to contract freely during tetanic contraction.

Hunterlab Color Reflectance

In this experiment, electrical stimulation did not affect the color of the semitendinosus after 30 min of bloom time (Table 2). Unruh et al. (1986) found that low voltage electrical stimulation of the longissimus dorsi produced steaks that were a brighter red than the control

longissimus dorsi; however, this was following days of display conditions and not anaerobic ageing.

Collagen Solubility

There were no significant differences between the control and stimulated muscles for collagen characteristics within a time period (Table 3). There was, however, a significant difference in soluble collagen between prerigor (0.05 h) and postrigor (168 h) for both the control and stimulated muscles (P < 0.05), yet there was no significant decrease in solubility as a percent of total collagen. Collagen solubility is a more accurate indicator of tenderness than absolute collagen content (Bailey 1985). In this experiment, liquid nitrogen was used to freeze samples. Preliminary trials (data not shown) showed that this did not change the solubility of the collagen. This does not agree with Jeremiah et al. (1980) who found that freezing samples with liquid nitrogen reduced collagen solubility.

There were significant correlations among the prerigor and postrigor collagen characteristics (P < 0.05). Prerigor total collagen was correlated to postrigor soluble, insoluble and total collagen (R = 0.53, 0.69 and 0.70, respectively). Correlations between the collagen characteristics and shear force indicated that collagen characteristics were not significantly related to the peak shear force. However, significant correlations of prerigor collagen to that found postrigor may be of value in predicting collagen characteristics of aged meat through a biopsy either shortly before or after death.

Table 2. Effect of electrical stimulation on Hunterlab color reflectance of excised semitendinosus muscles;

Reflectance	Control		Stimulated		SEM	
	24 h‡	168 h§	24 h	168 h	24 h	168 h
CA	15.63	17.30	14.54	16.73	1.10	0.66
CB	10.09	11.40	9.60	11.09	1.10	0.66
CL	33.12	33.98	32.61	34.17	1.10	0.66

[†]High CA values indicate increased redness, low CB values indicate increased blueness and high CL values indicate increased brightness for daylight (C).

 $[\]ddagger n = 9$ and n = 7 for control and stimulated groups.

 $[\]S n = 12$ for each control and stimulated group.

1.69

SEM Control Stimulated Collagen 0 h† 0 h 168 h 0 h§ 168 h fraction 168 h‡ Soluble 2.42a1.85b1.93a1.63b0.18 0.17 0.17 17.44 15.97 13.86 13.29 2.18 2.09 Insoluble¶ 2.09 2.22 2.13 19.87 17.82 15.79 14.92 Total ¶ 2.13 1.77 1.69 % soluble 14.70 11.88 12.91 10.96

Table 3. Effects of electrical stimulation on the collagen solubilities (g kg⁻¹ protein) of excised semitendinosus: pre- vs. postrigor values

Table 4. Effects of electrical stimulation on protein solubilities of excised semitendinosus: pre- vs. postrigor†

Protein fraction	Control		Stimulated		SEM	
	0 h	168 h	0 h	168 h	11‡	12‡
Sarcoplasmic§	511 <i>a</i>	192 <i>b</i>	541 <i>a</i>	185 <i>b</i>	10	10
Myofibrillar§	158a	275c	91 <i>b</i>	300c	20	20
Total soluble§	667a	467 <i>b</i>	632 <i>a</i>	489 <i>b</i>	20	20
% sarcoplasmic (of total soluble)	77.4 <i>a</i>	41.5 <i>c</i>	85.7 <i>b</i>	38.3c	2.36	2.26
% myofibrillar (of total soluble)	22.6a	58.5 <i>c</i>	14.3 <i>b</i>	61.7 <i>c</i>	2.36	2.26
Total protein¶	84.4	83.1	84.8	81.3	0.02	0.02

 $[\]dagger 0$ values have n=11 control, n=12 stimulated. 168 h values have n=12 for control and stimulated muscles. $\pm \text{Sample size}$

Muscle Protein Solubility

Prerigor protein solubility indicated inherent solubility differences between the control and stimulated muscle groups (Table 4). The control muscles prerigor had significantly (P < 0.05) less sarcoplasmic proteins extracted as a percent of the total soluble protein than the stimulated group. The control muscles also had significantly more soluble myofibrillar proteins prerigor, as g kg⁻¹ of protein and as percent of total soluble protein, than the stimulated muscles (P < 0.05), indicating that inherent differences existed between the two groups. The stimulated group had a significantly greater increase in myofibrillar protein solubility during ageing (P < 0.05) than the control group (47.5 vs.

35.6% for stimulated and control groups, respectively). Interestingly, there were no differences between the control and stimulated muscles following ageing nor any significant correlations between pre- and postrigor protein solubilities, suggesting that the prerigor solubility of muscle proteins was unrelated to meat tenderness and was not indicative of the protein solubilities found postrigor. Including the prerigor variation in myofibrillar protein solubility using an analysis of covariance revealed that there was no significant effect of treatment. Therefore, the significant difference between the slopes of the myofibrillar solubility curve during ageing, as indicated by the significant interaction between stimulation and time, was due to prerigor

 $[\]dagger n = 11$ and 12 for control and stimulated groups, respectively.

 $[\]ddagger n = 12$ for control and stimulated groups.

[§]Standard errors of means for control and stimulated, respectively.

[¶]g collagen kg⁻¹ protein (N \times 6.25) basis.

a,b Values with different letters in rows are significantly different (P < 0.05).

[§]g/kg protein of muscle sample.

 $[\]P(N \times 6.25) \times 100 = \%$ total protein.

a-c Values with different letters are significantly different (P < 0.05).

differences alone and not an effect of treatment. The decrease in the solubility of total protein in the muscles from pre- to postrigor (Table 4) was unexpected, but not inexplicable. Degradation of sarcoplasmic enzymes of the muscle (Scopes and Lawrie 1963) would reduce the protein in the sarcoplasmic extraction as well as in the total extractable portion using the method of Link et al. (1970).

CONCLUSIONS

The tenderness of unrestrained muscle was not changed with the use of low voltage electrical stimulation, despite a significantly faster rate of pH decline and lactate accumulation than the control group, indicating that an increased rate of glycolysis postmortem may not be the most important factor in improving tenderness during ageing. Indirectly, the data suggested that restraint of muscle may be required during low voltage electrical stimulation to increase tenderness through structural damage. Prerigor measurements of collagen content were correlated to those found postrigor; however, protein solubilities were not, primarily due to changes in sarcoplasmic protein solubility.

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