The effects of electrically induced live-animal muscle contraction on bovine muscle glycogen

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Introduction

Meat obtained from intact male cattle is darker in color and less tender than meat obtained from castrated male cattle (Jeremiah, 1978; Seideman et al., 1982; Crouse et al., 1983). The dark color of meat obtained from intact males has been largely associated with antemortem stress usually resulting from exhausting exercise or fasting (McVeigh and Tarrant, 1982a, 1982b).

At present, the most effective method of experimentally inducing stress is by excitement and physical activity. This method is not easily reproducible or controlled and is time consuming. Electrical immobilization causes muscle contraction and release of hormones associated with stress (Macfarlane, 1981). Electrical immobilization may reduce muscle glycogen content independently or when interacting with exogenous epinephrine as a treatment that could be developed to mimic mental and physical stress related to social interaction or handling. This procedure would provide a useful method of studying animal stress under controlled conditions. Therefore, the objective of this study was to study the effects of exogenous epinephrine and electrical immobilization on bovine muscle glycogen concentration.

Materials and Methods

Design. Three trials were conducted utilizing Angus x Hereford steers. In trial 1, 11 steers weighing 523 kg (SD = 24 kg) were randomly assigned to a control group (5 steers) or an electrical immobilization (EI, 6 steers). Trials 2 and 3 utilized 12 or 16 steers, respectively, weighing 414 kg (SD = 44 kg). Steers were randomly assigned in equal numbers to control, EI, exogenous epinephrine or EI plus exogenous epinephrine. Response variables were observed on biopsied muscle taken 24 h pretreatment, .5 h post treatment and 24 h post treatment in trial 1. Biopsies were taken 24 h post treatment in trials 2 and 3.

Animals were fed to appetite an 84% TDN diet containing corn silage (IFN 3-08-153), corn (IFN 4-02-931), soybean meal (5-04-604) and urea for at least 2 mo before and during the experiment. Control steers were handled similarly to treated groups.

Treatments. The Stockstill[1] apparatus was used to electrically immobilize the steers. A needle electrode was placed subcutaneously in the vicinity of the animal's tailhead and another electrode clamped to the animal's jaw to administer the current. The maximum electrical current produced was 55 volts at 240 milliamps; however, after initial administration at about 75% of the maximum, the current was reduced to about 60% of maximum as determined on an individual animal basis as monitored by respiration.

In trials 1 and 2, current was applied on a continuous basis for 15 min. In trial 3, after each 25 sec of continuous use the Stockstill was turned off for 5 sec for a total on and off time of 15 min. In trials 2 and 3 epinephrine (5 mg/ml) was administered subcutaneously (13.2 mg/100 kg live-animal weight).

Biopsy and Glycogen Assay. A needle biopsy procedure (Tarrant and McVeigh, 1979) was used to obtain longissimus muscle samples on the right and left sides of the animal between the first and fifth lumbar vertebra. A local anesthesia (procaine hydrochloride, 2.5% w/v) was used. About 1.5 g sample was removed from the animal, large pieces of adipose or connective tissue were rapidly excised and the sample was frozen in liquid nitrogen.

Glycogen was assayed by the procedures of Bergmeyer and Bernt (1974). Samples were homogenized using a homogenizer in 5 parts (w/v) of .6N perchloric acid. After centrifugation at 10,000 x g for 15 min, a 200 µl aliquots of the supernatant fraction was incubated with amylglucosidase for 2 h at 40 C to degrade glycogen to glucosyl units. Total glucose and free glucose were determined in separate 200 µl aliquots of supernatant fraction by Gilford Diagnostics Glucose (HK) kit[2]. The difference between total glucose and free glucose was considered glycogen glucose. Glycogen was expressed as µmol glycogen-glucose/g of sample. Lactate and glucose-6-phosphate (G-6-P) were measured in perchloric acid extracts by established procedures (Hohorst, 1965; Bergmeyer and Bernt, 1974).

Results and Discussion

Trial 1. Least-squares means for the control and EI steers over the three time periods are given in table 1. Time periods are for 24 h pre-EI, .5 h post-EI and 24 h post-EI. Mean values for control and EI steers were similar. This similarity was constant over the three time periods. Quantities of glycogen observed in control and EI steers are very similar to those previously reported by McVeigh and Tarrant (1982b). These data indicate that EI has no immediate (.5 h) or long-term (24 h) effect on longissimus muscle glycogen content.

Trials 2 and 3. Least-squares means for traits observed for the EI and epinephrine treatments in trials 2 and 3 are given in table 2. Significant glucose (P<.10) and lactate (P<.05) trial x treatment interactions were observed. In trial 2, where glucose content was high in the control animals, glucose, lactate and G-6-P content were decreased at 24 h after injection of epinephrine. Trial 3 animals had substantially lower glucose content initially and in these steers EI and epinephrine actually tended to increase glucose and lactate concentrations by 24 h post-treatment. Because EI had no significant effect on glycogen content in either trial, it is not apparent how it affected glucose and lactate content in trial 3. It is possible that the small (10%) decrease in glycogen content elicited by EI in trial 3 steers was sufficient in magnitude to elevate glucose and lactate concentrations. The net reduction in metabolite levels in trial 2 steers indicates that the glucose carbon released from epinephrine-stimulated glycogenolysis was metabolized quite rapidly in the more youthful steers in trial 2.

Muscle glycogen content was greater (P<.01) in steers in trial 3 than in steers in trial 2, while glucose, lactate and G-6-P were lower (P<.01) in trial 3 steers (table 2). Because steers in both trials were the same breed, sex and fed the same diet, differences in muscle metabolite levels probably were associated with increased live-animal weights or length of time fed.

In Trials 2 and 3, epinephrine injections decreased (P<.01) glycogen content 30 to 35% (table 2). No significant EI x epinephrine treatment interaction was observed. Reduction in glycogen content in the present study is less than the 70% of the reduction observed by McVeigh and Tarrant (1982b) where two epinephrine injections (twice the dosage) were administered.

In summary, inducing antemortem muscle contraction with the Stockstill apparatus was ineffective in lowering muscle glycogen, so that this procedure would not replace extensive physical activity as a means of depleting muscle glycogen for experimental purposes. However, since the Stockstill provides prolonged animal immobilization and presumably analgesia without affecting muscle metabolism, it should serve as a suitable replacement for chemical anesthesia.

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[1] Feenix Corporation, Box 161, Lake Wylie, SC 29710.

[2] C&I Products, 1118 E. University, Des Moines, IA 50316.

TABLE 1. LEAST-SQUARES MEANS OF MUSCLE GLYCOGEN CONTENT FOR STEERS IN TRIAL 1[a]

Treatment	Period[b] -24	Period[b] .5	Period[b] 24 h	Residual SD
Control	69.6	71.6	74.2	13.5
Electrical immobilized	65.5	64.9	70.8	13.5

[a] Glycogen reported as µmole/g.
[b] Time of electrical immobilization was time 0 h.

TABLE 2. LEAST-SQUARES MEANS OF TRAITS FOR TRIAL X TREATMENT SUBCLASSES

Subclass	Live weight	Glycogen	Glucose	Lactate	Glucose-6-Phosphate
	kg	µmole/g	µmole/g	µmole/g	µmole/g
Trial:	**	**	**	**	**
2	370	54.6	6.11	11.69	1.43
3	446	67.2	1.72	8.46	.63
Treatment:		**			
Control	412	74.7[a]	4.26	9.42	1.15
Electrically stimulated (EI)	401	70.1[a]	4.47	10.98	1.10
Epinephrine (E)	411	51.4[b]	3.71	10.02	.97
EI+E	406	47.3[b]	3.20	9.88	.90
Trial x Treatment:					
Trial 2			†	*	
Control	360	69.4	7.04	12.24	.68
Electrically immobilized	371	67.5	7.06	12.32	.60
Epinephrine	375	44.7	5.71	11.32	.33
EI+E	372	36.9	4.63	10.86	.11
Trial 3					
Control	464	80.1	1.49	6.60	.63
Electrically immobilzed	433	72.6	1.89	9.63	.60
Epinephrine	447	58.1	1.71	8.72	.60
EI+E	440	57.8	1.77	8.90	.68
Residual standard deviation	24	8.2	.97	1.16	.30

† Trial x treatment interaction (P<.10).
* Trial x treatment interaction (P<.05).
** Means within a column within main effect differ (P<.01).
[a,b] Means with different superscripts differ.