LOW VOLTAGE ELECTRICAL STIMULATIONS OF BEEF CARCASSES : DISTRIBUTION OF TENDERIZING EFFECT IN THE CARCASS AND RELATION TO CHANGES IN SARCOMERE LENGTH

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INTRODUCTION

Application of an electrical potential (and current) to beef carcasses or sides (electrical stimulation or ES) shortly after slaughtering has received considerable attention in meat technology. The increased tendency to cool carcasses as soon as possible after slaughter and eventually, after removal of bones (hot boning) and vacuum packing of the cuts, is related to this interest. ES is indeed known to accelerate glycolysis, depletion of ATP, fall of pH and installation of rigor mortis enabling rapid cooling without toughening due to cold shortening (for review see Bendall, 1980). Although there is a general agreement on the principle there is considerable contradiction with regard to the optimal electrical parameters (e.g. AC or DC, voltage, frequency, DC pulse width, time of stimulation etc.) to be used in stimulation. Also tenderization by ES is not always associated with longer sarcomeres, which led several workers to suggest a tenderizing effect of ES other than the prevention of cold shortening (Will et al, 1979; Bouton et al, 1978; Vandekerckhove & Demeyer, 1978). Results on sarcomere length however may be different depending on the use of microscopic or diffraction techniques, whereas immediate post slaughter temperature conditions may affect the tenderizing effect of ES (George et al, 1980; Bouton et al, 1980). Tenderization may be related to the more rapid action of proteolytic enzymes under the different temperature/pH conditions of stimulated muscles compared to non-stimulated muscles (Bendall et al, 1976). Also, a faster releases of lysozomal enzymes (Dutson & Yates, 1978) and physical disruption of the muscle fibers (Savell et al, 1978) may be involved.

In earlier work, we proposed a low voltage ES method (Vandekerckhove et al, 1978) to prevent cold shortening, but obtained evidence that tenderization apart from the prevention of cold shortening occurred (Vandekerckhove & Demeyer, 1978).

In this paper, we report results on the effect of low voltage ES on tenderness and sarcomere length of muscles removed immediately after slaughter (hot boned), vacuum packed and chilled at ca. 1°C. Results varied between individual muscles. In order to assess more fully the effect of low voltage stimulation, tenderness and sarcomere length were then measured on 21 different muscles removed from control and stimulated sides, cooled immediately after slaughter and ES during 1 week.

MATERIAL AND METHODS

Animals and treatment of carcasses

All experiments were done with 7 bulls (mean values ± S.E. for live weight : 485 ± 25 kg; dressing % : 61.8 ± 1.0%; age : 13.5 ± 1.3 m) of Belgian breeds. The animals were slaughtered in the slaughterhouse of our laboratory, after stunning using a captive bolt pistol and pithing. After slaughtering and dressing, the carcasses were split into sides, the whole process lasting 45 min. The experiments are summarized in table 1. The first experiments involved boning on the rail of the left carcass sides into 22 cuts immediately after slaughtering according to Brasington & Hammons (1971). Boning lasted 73 ± 7 min (mean value ± S.E.) and was preceded by ES (expt. 3 & 4) or not (expt. 1 & 2). After vacuum packing in polyamide laminated polyethylene (Sidamil X- type EAK 141 - Sidac - Ghent), cuts were cooled for 1 week at ca 1°C. Control right sides were cooled immediately after slaughtering (expt. 1 & 3) or after 24 h conditioning at 15°C. ES was carried out using the top half of A.C. giving pulsed D.C. of 50 Hz with a pulse width of 10 msec. Average currents (RMS) of 100 mA, 200 mA and 600 mA were applied consecutively for 80 sec. each, giving voltages of 100-150 V (RMS) equivalent to 200-300 V (peak) (V RMS = V peak $\times \sqrt{\frac{Q}{200}}$ with Q = frequency in Hz according to Bendall, 1978). The apparatus and electrodes described earlier were used (Vandekerckhove et al, 1978). A 4 needle electrode was placed in the Semitendinosus and a long single needle in the neck. Experiments 5, 6 and 7 involved immediate cooling of stimulated left sides together with control right sides.

DETERMINATIONS - 1. In expts. 5 to 7 temperatures and pH were measured at regular intervals after slaughtering (10 measurements between 1 and 24 h after slaughtering), on sites corresponding to muscles no. 2,3,4,5,7 and 9. Each value of pH was a mean calculated from at least 3 measurements giving similar values, obtained by insertion of an Ingold pointed combined electrode, attached to a digital pH meter (Knick portamess 651, Knick, Berlin, BRD) into a fine slid

made with a knife. Temperature (^{o}C) was read only once by insertion of a thermocouple (5 cm), using a digital instrument (Technoterm no. 9503, Technoterm, Lenzkirck, BRD). Similar measurements were made on all cuts in expts. 1 to 4, immediately before vacuum packing (2 h after stunning).

2. Sarcomere lengths (SL) were determined microscopically (SL_m) and/or by laser diffraction (SL_1). The latter method involved fixation of a thin slice of muscle (1 - 2 g obtained from the larger slice used for shear force determination) as described in the laser manual (Spectra physics no. 145-02 laser 1.5 mW, He-Ne, 632.8 nm, Spectra physics, Mountain View, USA) and measurement of diffraction pattern, using ca. 20 fixed muscle fibers (Ruddick & Richards, 1975). For microscopic determination, a suspension of fresh muscle was prepared as described by Davey & Gilbert (1974) and a total of ca. 150 sarcomeres was counted. The average ratio SL_m/SL_1 was 0.99 ± 0.10(mean value $\pm$ S.D. for 56 comparisons) (unpublished data).

3. shear-force (kg) was measured using a Warner-Bratzler cell and an Instron Universal Testing Machine (Instron Ltd, High Wycombe no. 1640) on cooked meat samples. From each muscle after removal of fat and connective tissue, a slice was obtained using a double bladed knife, perpendicular on the muscle fiber direction (thickness ca. 3 cm). The samples were put in polyethylene bags and submersed in water ($75^{o}C$, 1h). After cooling, a maximum of ca. 50 cork bore samples (diam. 1.25 cm) parallel to the muscle fibers was obtained and used for shear force determination. Small muscles were used completely, giving less than 50 cork bore samples.

Statistical evaluation - Stimulated and control muscles were compared using the paired t-statistic. In order to evaluate the effect of stimulation on the whole side, weighed average shear force values were calculated for the 21 muscles, using weight factors derived from the physical weight and commercial value of the muscles sampled (table 2). Using the weight factors, a weighed paired t-statistic was calculated, by entering a data pair for a particular muscle a number of times, related to the total weight factor for that particular muscle. In calculation of t the real number of pairs were used (R. Moermans, 1980).

RESULTS

Experiments involving hot boning and ES - ES did not greatly affect average pH or temperature of hot boned cuts. Without ES, mean values $\pm$ SD were 6.6 ± 0.2 and $34.6 \pm 3.7^{o}C$. (expt. 1 and 2) respectively, with ES 6.3 ± 0.2 and $34.4 \pm 4.8^{o}C$.

Table 3 shows that compared to cooling on the carcass, there was a tendency for tougher meat after hot boning and vacuum packing (expt. 1) and this was prevented by ES (expt. 3). Compared to conditioning on the carcass however hot boning gave tougher meat (expt. 2) even after ES (expt. 4). The calculation method used to characterize the effect of ES on the whole side gave no significant effect however. Fig. 1 illustrates the relative changes of shear force and SL (expressed as ratios treated/control) for the 6 muscles sampled. Toughening of meat expt. 2 is clearly related to cold shortening. It is striking however that although ES tended to overcome this effect, no change in sarcomere shortening was involved: whatever the treatment, hot boning resulted in muscles with shorter sarcomeres when compared to an intact carcass. This is a first indication of tenderization by ES not involving changes in SL (e.g. muscle no. 5 in fig. 1). As differences in response, between the muscles sampled were apparent (e.g. expt. 4 in fig. 2) it was decided to investigate more in detail the effect of low voltage ES on various muscles representing ca. 50% of total muscle (table 2).

2. Experiments involving ES only - The effect of ES on rate of cooling and rate of pH fall was assessed by calculation of mean values for the 6 carcass sites measured at each measuring time. Variation coefficients were ca. 8% and 2% for temperature and pH respectively. Rates of cooling and pH fall were estimated by regression of these means against time.

For rates of cooling regression following $y = a \cdot e^{-bx}$ was used, for rates of pH fall, the highest determination coefficient was obtained using $y = a + \frac{b}{x}$. The latter equations were then used to estimate the temperature immediately after ES (t_{ES}) ($x = 0.75$ h in $y = a \cdot e^{-bx}$) and the time after stunning at which pH 6 is reached[13] (t_6) ($y = 6.0$ in $y = a + \frac{b}{x}$). Data in table 4 show that carcass temperature was consistently increased by about $1^{o}C$ after ES, whereas pH was about 0.6 units lower than control sides 25 min. after ES.

The average time after stunning necessary to reach pH 6.0 was reduced from 4.8 h to 2.0 h. As shown in table 5 the latter effect was evenly distributed over the sites measured and ES apparently reduced between animal variation. The effect of ES on shear force and sarcomere length is shown in fig. 2 : there is a clear tendency for a decrease in shear force values due to ES (shear force ratio ES/control <1) but this tendency is not related to longer sarcomeres. The tenderizing effect is evenly distributed over the various muscles studied (table 6). From an evaluation of the effect on the whole carcass side, taking into account weight and commercial value of the muscles studied weighed average tenderization of 6% can be calculated (table 7). No statistical significance was obtained although fig. 2 clearly demonstrates the effect.

DISCUSSION

The data obtained demonstrate that hot boning followed by vacuum packing and cooling results in tougher meat confirming earlier work (Buchter, 1977). This effect is more or less outspoken when compared to conditioning on the carcass or to immediate cooling of the carcass respectively. Low voltage ES has a tendency to overcome this toughening effect but this was not associated with longer sarcomeres.

The method of measurement of sarcomeres does not effect this conclusion as SL changes measured by microscope or by diffraction were clearly related (fig. 3).

The effect of ES on shear force values did not seem to be evenly distributed over the 6 muscles studied (expt. 4, fig. 1) but this was not due to an uneven distribution of the current, as indicated by experiments were muscles were kept on the carcass. These experiments showed that low voltage ES increased rate of pH fall so that pH 6 was reached within about 2 h after stunning or a saving of 58%. It is noteworthy that 2 h is similar to the value obtained by Bendall et al (1976) using 700 V (peak) stimulation. The non stimulated sides however in the latter experiments using heifer carcass sides took over 8 h to reach pH 6. Recent work by Bouton et al (1980) also indicates that ES at 110 V (peak) enables pH to fall to 6 within 2 h after stunning.

An evenly distributed overall tenderizing effect of ca. 6% was obtained by ES in our experiments, not associated with longer sarcomeres. As carcasses were cooled immediately after ES, before pH 6 was reached (table 4) both control and stimulated carcasses probably suffered cold shortening (Bendall, 1980).

This strongly indicates that ES tenderizes meat by a mechanism other than the prevention of cold shortening as suggested earlier (Demeyer & Vandekerckhove, 1978). This finding contrasts with other recent data (George et al, 1980; Bouton et al, 1980). The latter results however were obtained in different conditions. George et al (1980) conditioned the carcasses for 8 h at 16°C before cooling whereas Bouton et al (1980) used conditioning for 2 h at 12°C. Furthermore, the latter authors determined shear force and sarcomere length after a cooling period of only 22 h. Both groups also used a smaller number of muscles in evaluating the effect of ES on tenderness. According to Jones et al (1980) the different temperature/pH conditions for ES muscles are responsible for their increased tenderness and they state that when cooled below 10°C in the next few hours after ES no quicker tenderization is observed. Again this is in contrast with our results, where cooling was started immediately after ES. Nevertheless ES and control muscles also have a different temperature/pH history in our experimants also and this may well be responsible for the tenderization observed.

In summary, our results indicate that ES at 100 - 150V (RMS) 45 min. after stunning and immediately followed by cooling is sufficient to allow pH to be lowered to 6.0 within 2.5 h after stunning.
Carcass meat obtained 1 week after cooling is ca. 6% more tender due to ES but this is not reflected in a change of sarcomere length.

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REFERENCES

Bendall J.R., C.C. Ketteridge & A.R. George 1976 j. Sci. Fd. Agric. 27, 1123.
Bendall J.R. 1978. Personal communication
Bendall J.R. 1980. The electrical stimulation of carcasses of meat animals (manuscript in press).
Bouton P.E., A.L. Ford, P.V. Harris & F.D. Shaw 1978. J. Food Sci. 43, 1392.
Bouton P.E., A.L. Ford, P.V. Harris & F.D. Shaw 1980. Meat science 4, 145.
Brasington C.F. Jr. & D.R. Hammons 1971. ARS Bull. 52-63.
Brown A.J., M.E. Coates & B.S. Speight 1978. A photographic guide to the muscular and skeletal anatomy of the beef carcass. ARC - Meat Res. Inst. - Langford - Bristol - U.K.
Buchter L. 1977. 23d. Europ. Meet. Meat Res. Work. - Moscow - Russia.
Davey C.L. & K.V. Gilbert 1974. J. Fd Technol. 9, 51.
Dutson T.R. & L.D Yates 1978. Proc. 24th Europ. Meet. Meat Res. Work. - Kulmbach - Germany.
George A.R., J.R. Bendall & R.C.D. Jones 1980. Meat science 4, 51.
Moermans R. 1980. Personal communication.
Ruddick J.E. & J. F. Richards 1975. J. Food Sci. 40, 500.

Savell J.W., T.R. Dutson, G.C. Smith & Z.L. Carpenter 1978. J. Food Sci. 43, 1606.
Vandekerckhove P. & D. Demeyer 1978. Proc. 24th Europ. Meet. Meat Res. Work. - Kulmbach - Germany.
Vandekerckhove P., J. Hoozee & D. Demeyer 1978. Euro-Vee-Vlees 16,2.
Will P.A., R.L. Henrickson, R.D. Morrison & G.V. Odell 1979. J. Food Sci. 44, 1646.

Fig. 1 : Ratio of shear force and sarcomere length hot boned/control value for 6 muscles. Effect of low voltage ES (For details of experiments see table 1).

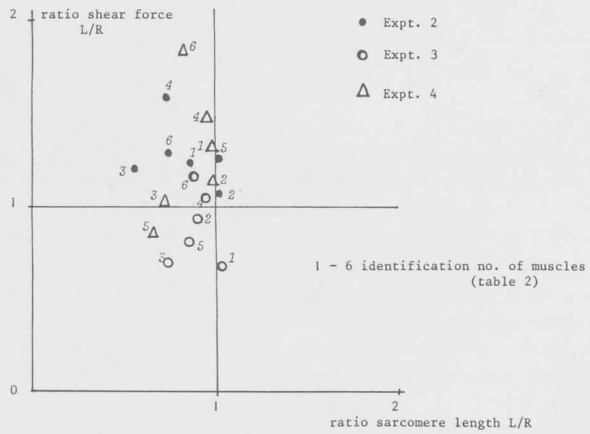

Fig. 2 : Change in shear force and sarcomere length by low voltage ES in 21 different muscles (3 experiments)

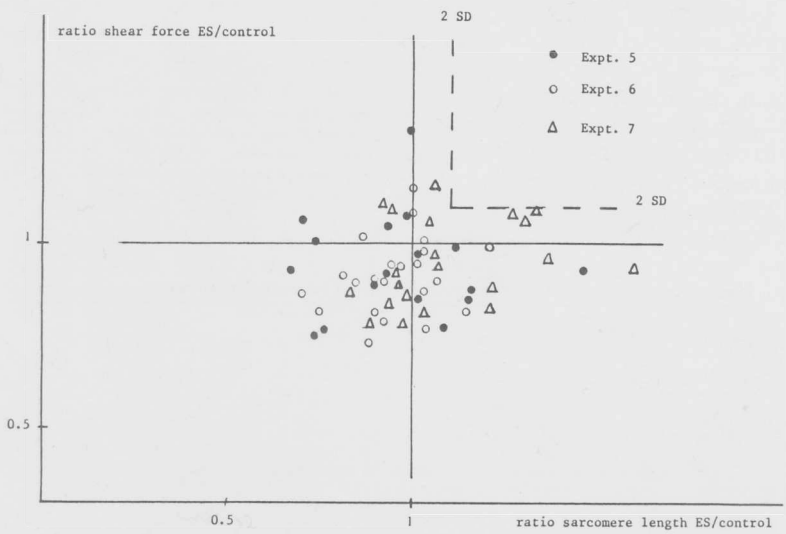

Fig. 3 : Relation between change in sarcomere length (SL) measured by microscope $(ES/control)_m$ and laser $(ES/control)_l$

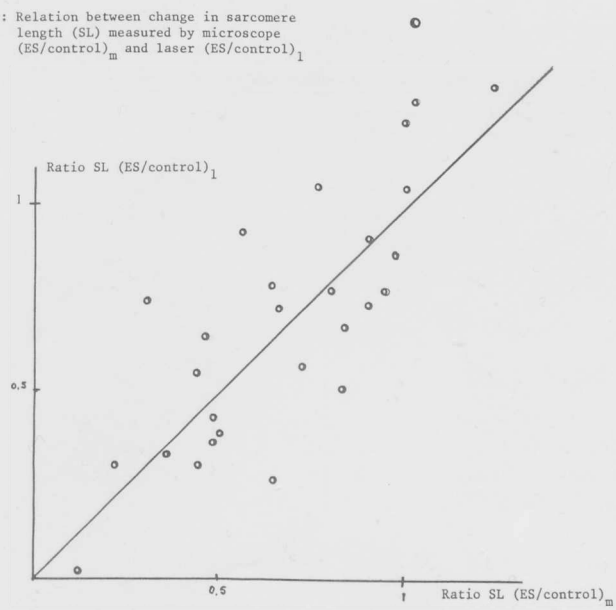

TABLE 1 : EXPERIMENTS INVOLVING HOT BONING AND ES

Treatment	Expt.1		Expt.2		Expt.3		Expt.4		Expt. 5-7	
	L[3]	R[3]	L	R	L	R	L	R	L	R
Electrical stimulation	-	-	-	-	+	-	+	-	+	-
Conditioning (24 h - 15°C)	-	-	-	+	-	-	-	+	-	-
Hot Boning	+	-	+	-	+	-	+	-	-	-
Vacuum packing	+	+[1]	+	-	+	-	+	+[1]	-	-
Cooling [2]	+	+	+	+	+	+	+	+	+	+

[1] The sides were cooled for 24 - 28 h at 2°C followed by deboning, vacuum packing of the cuts and further cooling.

[2] One week at 2°C (6 days for expt. 1 R and expt. 4 R)

[3] Left (L) or Right (R) sides

TABLE 2 : CALCULATION OF WEIGHT FACTORS FOR VARIOUS MUSCLES SAMPLED TO CHARACTERIZE A CARCASS

No.	Muscle	% of total muscle weight (1)[1]	Muscle weight factor[2]	Commercial value[3]	Commercial weight factor (2)	Total weight factor[4]
1	Long. dorsi(3-4)	3.34	1.00	S 1	1.14	19
2	Long. dorsi(6-7)	3.34	1.00	B 2	2.57	43
3	Tensor fasciae lat.	1.27	0.38	B 5	1.71	11
4	Semitendinosus	2.32	0.69	B 4	2.00	23
5	Infraspinatus	2.15	0.64	B 5/S 1	1.43	15
6	Gastrocnemius	1.95	0.58	B 3	2.29	22
7	Gluteus medius	3.71	1.11	B 2	2.57	48
8	Vastus intermed.	1.48	0.44	B 2	2.57	19
9	Triceps brachii c.m.	0.08	0.03	B 4	2.00	1
10	Semimembranosus	4.69	1.40	B 2	2.57	60
11	Pectoralis profund.	3.67	1.10	S 2	1.00	7
12	Biceps femoris	6.78	2.03	S 1	1.14	39
13	Splenius cervicis	0.60	0.18	S 2	1.00	3
14	Serratus ventralis	4.00	1.20	S 2	1.00	20
15	Extensor digit.lat.	0.23	0.07	S 2	1.14	1
16	Psoas major	0.37	0.11	B 1	2.86	5
17	Pectoralis superfic.	1.48	0.44	S 2	1.14	7
18	Latissimus dorsi	2.09	0.63	S 2	1.14	12
19	Subscapularis	1.19	0.36	B 2	2.57	15
20	Trapezius	1.31	0.39	S 2	1.00	6
21	Triceps brachii c.l.	3.06	0.92	B 4	2.00	31
	Total	49.11				

1 From Brown et al, 1978
2 Calculated by dividing % muscle weight by 3.34 (relative proportion of long. dorsi)
3 Meat to roast or grill was classified into 5 classes (B 1 down to B 5) and meat to stew or cook into 2 classes (S 1 down to S 2). Prices as multiples of S 2 are B 1 = 2.86, B 2 = 2.57, B 3 = 2.29, B 4 = 2.00, B 5 = 1.71, S 1 = 1.14.
4 Calculated as (1) x (2) x 5 and rounding off to the nearest integer.

TABLE 3 : DEVELOPMENT OF COLD SHORTENING AFTER HOT BONING, VACUUM PACKING AND COOLING. EFFECT OF LOW VOLTAGE ES (Shearvalues in kg)[1]

Muscle No.	Expt. 1		Expt. 2		Expt. 3		Expt. 4	
	L	R	L	R_c[2]	L ES[3]	R	L ES	R_c
1	4.56×	3.86	5.41×	4.36	3.68×	5.36	5.39×	4.02
2	3.82	3.94	3.62	3.35	4.36	4.79	4.05	3.60
3	-	-	4.99×	4.13	3.55×	5.06	5.23	5.15
4	4.41×	4.04	6.36×	3.97	5.60	5.41	6.69×	4.54
5	5.00×	3.72	5.57×	4.40	5.06×	6.24	4.19×	4.87
6	4.56	4.79	4.83×	3.72	5.73×	4.95	7.16×	3.85
Side mean[4]	4.33	4.07	4.88	3.85	4.72	5.19	5.33	4.14
Significance level[5]	N.S.		$p < 0.1$		N.S.		N.S.	

1 Left sides (L) are hot boned. Experimental details in table 1
2 R_c = conditioned right side
3 ES = low voltage ES
× significant difference for muscle studied
4 Weighed mean value calculated as described in the text
5 Corrected paired t-test (see Materials and Methods)

TABLE 4 : EFFECT OF LOW VOLTAGE ES ON RATE OF COOLING AND RATE OF pH FALL[1]

Expt.		Rate of cooling		Rate of pH fall	
		t_{ES} (°C)	b	pH_S[3]	t_6[4] (h)
5	Control	35.6	- 0.093 (94)[2]	6.96	5.31 (77)
	ES	38.4	- 0.094 (97)	6.15	1.74 (71)
6	Control	37.0	- 0.087 (99)	7.02	5.53 (90)
	ES	38.8	- 0.095 (99)	6.36	2.00 (93)
7	Control	37.4	- 0.086 (99)	6.69	3.57 (90)
	ES	38.0	- 0.086 (99)	6.36	2.29 (94)

1 Measurements of pH and temperature, assembled on 6 sites of the carcass sides were averaged (y) and fitted against time in hours (x) following $y = a.e^{bx}$ (temp.) and $y = a + \frac{b}{x}$ (pH).
2 ( ) : 100 R^2 of regressions
3 first measurement of pH after ES (25 min after ES)
4 time after stunning in which pH 6.0 is reached

TABLE 5 : EFFECT OF ES ON TIME AFTER STUNNING TO REACH pH 6.0 (HOURS) IN DIFFERENT MUSCLES (mean value ± S.E.)

Muscle	Control	ES
Long. dorsi (6-7)	5.8 ± 0.8	2.7 ± 0.1
Glut. Med.	4.3 ± 1.1	1.8 ± 0.2
Infraspinatus	5.7 ± 1.1	2.2 ± 0.3
Semitendinosus	4.7 ± 1.0	2.0 ± 0.2
Tensor fasc. lat.	6.3 ± 1.0	1.7 ± 0.1
Triceps brachii	4.1 ± 0.3	1.7 ± 0.2

TABLE 6 : RATIO OF SHEAR FORCES (ES/control) FOR VARIOUS MUSCLE GROUPS (mean value ± S.E.)

Muscle groups	Ratio of shear force (ES/control)
Leg	0.94 ± 0.03
Neck and Breast	0.92 ± 0.02
Shoulder	0.95 ± 0.06
Loin	0.92 ± 0.03

TABLE 7 : EFFECT OF LOW VOLTAGE STIMULATION ON WEIGHED MEAN SHEAR FORCE OF CARCASS SIDES

Expt.	Weighed mean Shear force (kg)		Significance of difference[1]
	Control (R)	ES (L)	
5	4.33	4.05	NS
6	4.41	4.06	NS
7	4.69	4.50	NS

1 Calculation explained in Materials and Methods