PE1.41 Muscle fiber types, capillary supply and enzyme activities in muscle of the pig. 269.00

Poul Henckel (1) poul.henckel@agrsci.dk, Erland Erlandsen(2), Niels Oksbjerg (3)
(1)Faculty of Agricultural Sciences
(2)Viborg Hospital
(3)Faculty of Agricultural Sciences

Abstract—Searching for causes of variation in meat quality characteristics between muscles information of the basic metabolic capacities of the individual muscles can be of importance. In the present paper we provide information from 18 different muscles, on fiber type distribution, fiber type areas, capillary supply and activity of marker enzymes for each muscle. Muscles samples were taken from 7 pigs. The myosin ATP-ase method was used to identify fiber types and the α-amylase PAS-stain to identify cell outlinings and capillaries. Stainings were all, performed on frozen sectioned samples of the muscles. Enzyme analysis were measured spectrophotometrical on homogenates from samples from the same location. The muscles in pigs cover the whole range of fiber type distribution from muscles almost completely comprising type IIB fibers to muscles almost completely comprised by type 1 fibres. Taken into consideration the metabolic characteristics of the fiber types this is reflected in the enzyme activity of the muscles. Capillary supply to type 1 and IIA are quite similar whereas the type IIB fibers display lower numbers. Considering the size of this fiber types this will have a important impact on its diffusion properties. A rather substantial variation is observed in the various parameters, which may be caused by the low number of animals but also a large variation in most morphological characteristics may have contributed substantially to the variation and this imply that the results should be interpreted with some caution.

F. A. Poul Henckel is with the University of Aarhus, Faculty of Agriculture, Dept of Food Science, Tjele, 8830. Denmark. phone: +45 89991239 fax: +45 89991564 (e-mail: poul.henckel@agrsci.dk) S. B. Erland Erlandsen is with the hospital in Viborg, Dept of Analytical Biochemistry, Viborg Hospital, 8800 Viborg, Denmark T. C. Niels Oksbjerg is with the University of Aarhus, Faculty of Agriculture, Dept of Food Science, Tjele, 8830. Denmark (e-mail: niels.oksbjerg@agrsci.dk)

Index Terms— capillaries, enzymes, fiber types, pig muscles, size

I. INTRODUCTION

The individual muscle fibre types in pigs as in other animals are characterised by a wide number of morphological and biochemical differences, which all are a reflection of the normal activity pattern of the muscle. Muscles primarily consisting of type I and IIA fibres are used for continuous work over prolonged periods of times, whereas muscles primarily consisting of type IIB fibres are used for sudden bursts of activity. Consequently frequency and properties of type I and type IIA fibres are more important in relation to daily physical activity and physical "welfare" of the animal than frequency and characteristics of type IIB fibres. Changes in characteristics of type I or type IIA fibres caused by treatments will thus have a greater impact on normal activity of the animals, than changes in characteristics of type IIB fibres In animals used for meat production size and number of fibres in a muscle has been associated to growth and growth potentials of the animal, whereas the capacity for energy production of the muscle fibres and muscles has been associated to meat quality. This, because of its importance for the post mortem pH development, which affects several important meat quality characteristics. Due to the characteristics of the individual fibre types, differences in growth potential and growth rate, as well as in meat quality may thus at least partly be explained by differences in fibre type distribution. In meat science most research has focussed on muscles that are economically important. The majority of these muscles, however, is characterised by a high proportion of type IIB fibres, which is the reason why much less information is available on the effect of treatments on other fibre types. Given the fact that fibre types also respond different to treatments it might be worthwhile to consider inclusion of muscles of diverging fibre type distribution in any investigation. Fibre type distribution in different muscles has been described earlier (1, 2), but comprehensive studies including associated traits like capillary supply, size of fibre types, and metabolic capacity of the muscles are lacking. Furthermore the general conclusion on

effects of a variety of treatments is based on information from muscles with low oxidative capacity, which this may not cover the effects occurring in high oxidative muscles. The purpose of this investigation is to describe the fibre type distribution, the size and the capillary supply of the fibre types and enzyme activity of marker enzymes of important metabolic pathways in different muscles of the pig

II. MATERIALS AND METHODS

Seven crossbred Danish Landrace x Danish Large White 4 females and 3 castrated males at 100 kg live weight were included in the investigation. The samples were taken from 30 minutes after slaughter and included the muscles M. Semitendinusus, M. Semimembranosus, M. Biceps Femoris, M. Vastus Lateralis, M. Vastus Medialis, M. Vastus Intermedius, M. Sartorius, and M. Soleus from the back part and M. Rectus Femoris, M. Longissimus Dorsi, M. Trapezius, M. Psoas Major, M. Supraspinatus from the trunk and M. Brachialis, M. Biceps Brachii, M. Triceps Brachii and M. Cleidocephalicus from the front part. For M. Longissimus Dorsi the samples were taken right above the last rib curvature, the other samples were taken at their geometrical center. All sampling was performed surgically. The excised samples were cut into two parts. The parts for biochemical analysis were immediately frozen in liquid nitrogen and the part for histochemical analysis were imbedded in OCT embedding media and frozen in isopentane cooled in liquid nitrogen. The sampled were stored at -800C until analysis. The biochemical measurements included measurements of the activity of citrate syntase (CS), ß-hydroxyacylCoA-dehydrogenase (HAD) and lactate dehydrogenase (LDH). Histochemically (10 µm serial sections) the samples were analysed for fibre type distribution, size of fibres types and capillary supply both to the individual fibres, as well as the muscle as a whole. Capillaries were visualized by the α-amylase PAS-stain (3) and the fibre types were identified according to Brooke and Kaiser (4) using the pH sensitivity of the myosin ATP-ase. Measurements were performed on at least 200 fibres. The morphometric analysis were carried out on an image analyse system designed specifically for muscle histochemical characteristics (TEMA, Scan Beam, Hadsund)(5). Measurements of enzyme activities were performed spectrophotometrically (Technicon RA-1000) according to principles of (6) and expressed on the basis of wet weight.

III. RESULTS AND DISCUSSION

In the present investigation we have used the classical ATP-ase method to identify individual fiber types. Methods based on antibodies for the different myosin isoforms are now possible, but not all antibodies are commercially available yet. Information of the state of the art on muscle fiber type identification in pigs is found in (7). There are different ways of calculating the distribution in fiber types. The obvious one, which is also the most commonly used, is in relative numbers. As, however, the individual fiber types most often differ in size, a more correct way from a physiological and functionally point of view is to express the distribution in relative area covered by the individual fiber types. In figure 1 and figure 2 is shown the distribution in fiber types by the two methods. This clearly shows that by using numbers one may overestimate the actual functional capacity of oxidative fibers in some muscles by approximately 25% somewhat depended on the fiber type distribution as an increase in fiber types I and IIA imply a more uniform size of fibers (Table 3) and consequently lesser degree of overestimation, eventually leading to an underestimation as observed in the vastus intermedius muscle. In table 1 is shown the activities of the marker enzymes CS (oxidative capacity) HAD (fat metabolism) and LDH (glycolytic capacity) these results are in line with what could be expected i.e. an increase in CS activity and HAD with increasing number of type I and type IIA fibers and a concomitant decrease in LDH activities. Capillary supply to the muscle is shown in table 2. There are two ways of describing capillarity in numbers and by diffusion where one takes into account the size of the fibers. As changes in activity level may affect both the size of the fibers and the capillary supply the most comprehensive picture requires measurements of both features. In the present text we have only used numbers, but diffusion capacities can easily be calculated from the values in table 2 and 3. For almost all variables was observed a rather large SD value. This is caused to some of extent by the low number of animals but the variation within muscles most likely constitutes the major part of the SD, which of course has an impact on the conclusions that can be made.

IV. CONCLUSION

The result show a vide variation in fiber type distribution in pig muscles from almost no type I1 fibers in the Semitendinosus to app. 80% in the Vastus Intermedius. This is reflected in the enzyme activities with higher oxidative and lower glycolytic capacity in muscles with larger areas or numbers of type I fibres. Capillary supply to type I and IIA are quite similar and lower supply is found for IIB fibers and no difference is observed between muscles Considering the relatively large SD of the values of course implies that the results should be interpreted with some caution. The results, however, still provide sufficient information to allow for a selection of muscles which covers varied distribution of fiber types for investigations.

ACKNOWLEDGEMENT

This investigation was funded by the Danish Research Council for Veterinary and Agricultural Science and the technical staff of our Laboratory is acknowledged for careful performance of the tedious analyses.

REFERENCES

[1] Kiessling, K.-H. & Hansson, I. (1983). Fibre composition and enzyme activities in pig muscles. Swedish Journal of Agricultural Research, 13, 257-261.

[2] Horák, V. (1988). Histochemical fiber type composition in 12 skeletal muscles of miniature pigs. Anat. Anz. , Jena 167, 231-238.

[3] Andersen, P. (1975). Capillary density in skeletal muscles of man. Acta Physiologica Scandinavica, 95, 203-205.

[4] Brooke, M.H. & Kaiser, K.K. (1970) Muscle fiber types: How many and what kind. Archives of Neurology, 23, 369-379.

[5] Henckel., P.., Ducro, B., Oksbjerg, N., & Hassing, L. (1998). Objectivity of two methods of differentiating fibre types and repeatability of measurements by application of the TEMA image analysis system. European Journal of Histochemistry, 42, 49-62.

[6] Lowry, O. H. & Passonneau, J. V. (1973) A flexible system of enzymatic analysis. Academic Press. New York.

[7] Lefaucheur,L., Milan, D., Ecolan, P. & LeCalennec, C. (2004) Myosin heavy chain composition of different skeletal muscles in Large White and Meishan pigs. Journal of Animal Science, 82, 1931-1941.

Table 1. Enzyme activities in different muscles of the pig mean values (mmol/kg/min) and standard deviations (LDH, lactate dehydrogenase, CS, citrate syntase, HAD, beta-hydroxyacylcoenzymeA-dehydrogenase)

Muscle	LDH		SD	CS		SD	HAD		SD
Semitendinosus	2273	±	465.30	5.03	±	1.14	4.20	±	0.51
Semimembranosus	3431	±	630.24	5.90	±	1.47	5.21	±	1.11
Rectus Femoris	2150	±	335.19	6.27	±	1.29	6.01	±	0.84
Biceps Femoris	2825	±	680.25	7.37	±	1.73	6.34	±	1.01
Longissimus Dorsi	3772	±	526.04	5.45	±	1.03	5.33	±	0.46
Brachialis	1873	±	440.28	12.08	±	3.99	11.40	±	3.53
Triceps Brachii	2130	±	575.41	8.61	±	1.12	7.81	±	1.66
Biceps Brachii	2104	±	578.83	8.57	±	1.56	8.08	±	1.49
Vastus Lateralis	2027	±	336.52	9.54	±	1.73	8.33	±	1.78
Vastus Medialis	2014	±	476.57	9.33	±	1.92	8.59	±	2.26
Psoas Major	2307	±	435.62	9.89	±	0.91	10.41	±	0.99
Gastrocnemius	2080	±	201.54	8.70	±	2.17	9.28	±	1.90
Sartorius	1958	±	570.06	9.38	±	1.49	10.10	±	2.99
Supraspinatus	1194	±	423.39	9.90	±	0.86	10.21	±	1.31
Soleus	1694	±	733.67	9.31	±	1.82	9.74	±	3.08
Cleidocephalicus	1618	±	347.10	10.76	±	1.44	12.17	±	3.08
Trapezius	888	±	247.44	11.99	±	0.86	17.07	±	0.58
Vastus Intermedius	363	±	60.09	14.60	±	2.91	18.01	±	1.14

Table 2. Number of capillaries surrounding the individual fiber types. Means and SD.

Muscle	Type I	SD	Type IIA	SD	Type IIB	SD
Semitendinosus	3.11	± 2.22	3.92	± 0.73	1.75	± 0.25
Semimembranosus	3.80	± 0.51	3.75	± 0.93	1.88	± 0.26
Rectus Femoris	3.68	± 0.38	3.33	± 0.53	1.55	± 0.28
Biceps Femoris	3.08	± 0.68	3.28	± 0.79	2.07	± 0.68
Longissimus Dorsi	3.52	± 0.35	2.97	± 0.89	1.53	± 0.25
Brachialis	3.87	± 0.62	3.93	± 0.35	2.84	± 0.47
Triceps Brachii	3.47	± 0.73	2.81	± 0.67	2.05	± 0.58
Biceps Brachii	3.74	± 0.69	3.65	± 0.63	2.17	± 0.47
Vastus Lateralis	3.50	± 0.39	3.22	± 0.72	2.05	± 0.54
Vastus Medialis	3.80	± 0.65	3.75	± 0.85	2.76	± 0.61
Psoas Major	3.88	± 0.27	3.35	± 0.56	2.06	± 0.26
Gastrocnemius	3.75	± 0.51	3.51	± 0.65	2.37	± 0.79
Sartorius	3.88	± 0.54	3.36	± 0.46	2.59	± 0.43
Supraspinatus	3.91	± 0.32	3.53	± 0.45	2.46	± 0.60
Soleus	3.50	± 0.39	3.23	± 0.76	2.47	± 0.56
Cleidocephalicus	4.02	± 0.70	3.37	± 0.70	2.64	± 0.44
Trapezius	3,67	± 0.68	2.77	± 0.48	2.14	± 0.26
Vastus Intermedius	3.25	± 0.26	2.90	± 0.46	2.75	± 0.42

Table 3. Mean area of the fiber types and mean fiber area (MFA) in μm^2

Muscle	MEANAI	SD	MEANAIIA	SD	MEANAIIB	SD	MFA	SD
Semitendinosus	4340	± 5	3386	± 824	4189	± 1087	4135	± 1049
Semimembranosus	2589	± 540	2874	± 1059	4541	± 1442	4391	± 1029
Rectus Femoris	1878	± 915	2446	± 821	3436	± 1156	3263	± 1063
Biceps Femoris	2534	± 664	2047	± 589	4344	± 1279	3926	± 1049
Longissimus Dorsi	2421	± 595	2308	± 446	3173	± 684	3013	± 592
Brachialis	2046	± 644	1953	± 689	4667	± 1359	4069	± 1042
Triceps Brachii	1947	± 664	2165	± 335	3251	± 768	2993	± 636
Biceps Brachii	2104	± 601	1720	± 598	3066	± 754	2790	± 634
Vastus Lateralis	1986	± 328	1885	± 622	2863	± 563	2604	± 482
Vastus Medialis	2003	± 411	2264	± 875	3423	± 907	2983	± 706
Psoas Major	2575	± 629	2716	± 846	3163	± 927	2969	± 829
Gastrocnemius	2120	± 574	1607	± 415	2832	± 574	2463	± 487
Sartorius	3225	± 984	3418	± 1288	4816	± 1386	4279	± 1233
Supraspinatus	1940	± 368	1825	± 466	3227	± 926	2688	± 716
Soleus	2728	± 600	2110	± 575	3969	± 1568	3562	± 1078
Cleidocephalicus	3393	± 826	2840	± 559	3844	± 892	3458	± 722
Trapezius	2972	± 1021	2118	± 611	2604	± 621	2678	± 765
Vastus Intermedius	2774	± 642	2498	± 675	2900	± 1119	2745	± 682

Figure 1. Fiber type distribution in relative areas in muscles of the pig.

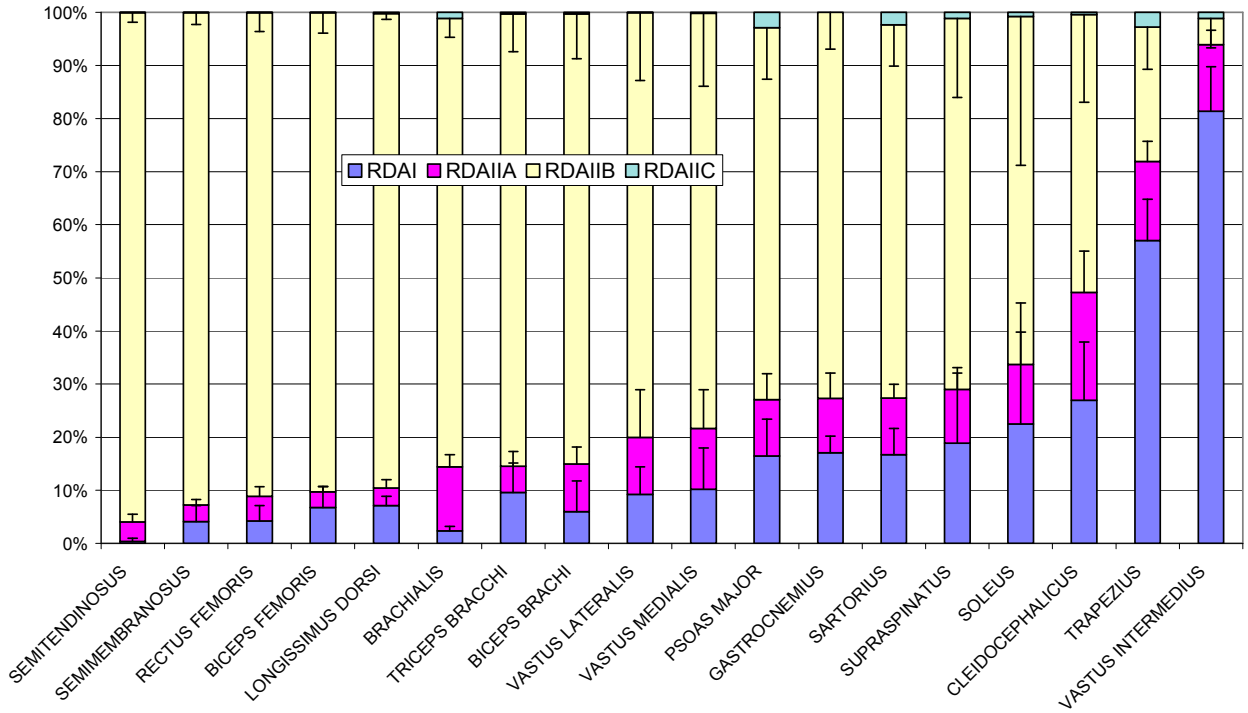

Figure 2. Fiber type distribution in relative numbers in muscles of the pig.