S5P10.WP

HISTORICAL AND BIOCHEMICAL MUSCLE PROPERTIES IN F_2 ANIMALS FROM CROSSES BETWEEN EUROPEAN WILD BOAR AND SWEDISH YORKSHIRE PIGS

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INTRODUCTION

Muscles from wild board show a greater oxidative capacity (Essén-Gustavsson & Lindholm, 1984), a larger myoglobin content (McCampbell *et al.*, 1974), and the same or larger cross-sectional area of type I and II fibres, on the basis of equal body weight (Rahelic and Puac, 1980-81; Szentkuti *et al.*, 1981; Bader, 1983) compared with domestic pig. A larger proportion of type I and smaller proportion of IIB fibre have been found in wild boar and crosses between wild boar and domestic pig, compared with the domestic pig (Rahelic and Puac, 1980-81; Essén-Gustavsson and Lindholm 1984; Rede *et al.*, 1986).

When comparing halothane genotypes in domestic pig, no differences in muscle enzyme activity or in fibre type composition have been found (Essén-Gustavsson and Lindholm, 1984; Lundström *et al.*, 1989). Larger muscle fibre cross-sectional areas have been seen in pigs homozygous for the halothane gene, compared with halothane-gene-free pigs (Wicke *et al.*, 1987; Essén-Gustavsson *et al.*, 1992). A recent study shows that in halothane-gene-free pigs the variation in histochemical and biochemical muscle properties was low in pigs selected for rapid lean tissue growth on two different protein diets (Karlsson *et al.*, 1993).

A reference pedigree for gene mapping by cross-breeding the European wild boar with the domestic pig of Swedish Yorkshire breeding, has recently been developed (L. Andersson *et al.*, unpublished). This pedigree provides an animal material of an expected large variation in several traits, with a continuum
from wild boar to domestic pig. By a systematic screening of the genetic markers on the gene-map, it is likely that associations between genetic markers and physiological traits will be found.

The aim of this investigation was to study histochemical and biochemical muscle properties in LD and BF from F_2 animals from crosses between European wild boar and Swedish Yorkshire pig, where muscle fibre properties are expected to have a greater genetic variation compared with domestic halothane-gene-free pigs. In addition, by screening among the genetic markers available, a marker-gene which was associated with histochemical and biochemical wild boar traits, was searched for.

MATERIAL AND METHODS

Animals

From a Swedish reference pedigree for gene mapping, 20 F_2 animals (castrates and gilts) were sampled from 200 F2 animals on the basis of daily growth rate from birth to slaughter (high or low) and the presence of absence of the halothane gene (Hal^NHal^N or Hal^NHal^n). One of the two wild boars in the parental generation, was found to carry the halothane gene (Hal^NHal^n). The animals were fed 90% of the Swedish standard feeding regimen (16% crude protein, 12.2MJ/kg).

The animals were slaughtered the week their live weight reached 80kg or at a maximum age of 190 days. They were transported 5km from the research station to the abattoir and, without lairage, they were electrically stunned with low voltage (90V, 0.8-1.0A, 15s) using a restrainer.

Genetic markers

More than 100 genetic markers have been analyzed on these animals for the purpose of developing a porcine linkage map (Ellegren *et al.*, 1993). Information on two of these markers was selected for the present study. The ATP1B1 locus, encoding for the porcine Na^+/K^+-ATPase was included, since Na^+/K^+-ATPase activity has been shown to be associated with oxidative metabolism in the skeletal muscle (Knochel *et al.*, 1985; Kjeldsen *et al.*, 1988; Kjeldsen *et al.*, 1990); the three allele ATP1B1 RFLP was analyzed as recently described (L. Marklund *et al.*, submitted). The CRC locus, for Calcium Release Channel, was also included since the halothane mutation in this gene is well known to have a great impact on carcass lean tissue content and muscle metabolism in pigs (e.g. Webb *et al.*, 1982; Lundström *et al.*, 1989). The point mutation at codon 615 controlling the halothane reaction (Fujii *et al.*, 1991) was typed using an allele-specific PCR method (L. Andersson *et al.*, unpublished).

Muscle samples

Muscle samples for histochemical and biochemical analyses were taken 30 minutes after exsanguination from the LD at the last rib and from the central part of BF, frozen in liquid N_2 and stored at -80°C until analysis.

Histochemical analyses

Serial transverse sections (10µm) were cut in a cryostat at Ä20°C and stained for myofibrillar ATPase after preincubation at pH 4.6, and classified as type I, IIA and IIB (Brooke and Kaiser, 1970). Glycogen content in muscle fibres was evaluated on 20µm thick transverse sections stained for glycogen with the periodic acid-Schiff method (PAS; Pearse, 1961). Fibres were classified as glycogen depleted when unstained, and they were also identified according to fibre type. A computerized image analysis system designed for muscle fibre analysis (BIO-RAD Scan Beam, Hadsund, Denmark) was used to calculate the proportions (%) and mean fibre cross-sectional area (µm²) for each fibre type. A muscle sample of 150 fibres were used in the analyses.

Biochemical analyses

On 1 to 2mg of freeze-dried muscle, which was dissected free from blood, fat and connective tissue under microscope, the activities of the enzymes citrate synthase (CS), 3-OH-acyl-CoA dehydrogenase (HAD), and lactate dehydrogenase (LDH), was analyzed and expressed on a dry weight basis (Essén *et al.*, 1980; Essén-Gustavsson *et al.*, 1984).

Glycogen content was analyzed on 1 to 2mg of freeze-dried muscle which was boiled for two hours in 1M hydrochloric acid in sealed tubes, in order to form glucose residues. The concentration of glucose was measured fluorimetrically (Lowry and Passonneau, 1973) and glycogen content was expressed as glucose units on a dry-weight basis.

Statistical analysis

The data were analyzed by the least-squares method using the GLM procedure (SAS Institute Inc., 1989). The statistical model included the fixed effects ATP1B1 (Na^+/K^+-ATPase)-genotype (`wild' or `domestic') and halothane genotype (Hal^NHal^N or Hal^NHal^n). There were no significant interactions found between the effects. Least-squares means and standard error of the estimates are presented.

RESULTS AND DISCUSSION

Differences in muscle fibre properties between the two ATP1B1 genotypes were found in LD, where the `wild' genotype had a higher enzyme activity of HAD and larger fibre cross-sectional area for types I and IIB, compared with the `domestic' type (Table 1). No significant differences were found in LD between the ATP1B1 genotypes, but the tendencies were the same as in BF. This is in agreement with studies comparing wild boar and crosses between wild boar and domestic pig, with domestic pig (Rahelic and Puac, 1980-81; Bader, 1983; Essén-Gustavsson and Lindholm, 1984; Rede *et al.*, 1986). The marker for the Na^+/K^+-ATPase-gene, used in the present study, seems to be linked with the physiological characteristics muscle fibre cross-sectional area and lipid oxidation. It has been found that a high oxidative capacity in muscle is related to a high activity and content of Na^+/K^+-ATPase in the dog (Knochel *et al.*, 1985), rat (Kjeldsen *et al.*, 1988) and in human (Kjeldsen *et al.*, 1988; Kjeldsen *et al.*, 1990).

Among the 20 F_2 animals 10 were carrying the halothane gene (Hal^NHal^n). These animals had in the BF larger mean fibre areas for type I (3586; 270) and IIA fibres (3217; 231), compared with halothane-gene-free pigs (2702; 263 and 2540; 225 respectively). In the LD, no significant halothane genotype differences in these traits were found, but the tendencies were the same as in BF. The larger muscle fibre area seen in and pigs carrying the gene, is confirmed by results from a study where the two halothane-homozygote genotypes was compared (Essén-Gustavsson *et al.*, 1992). When selection for a high lean meat content and large cross-sectional area for type IIB fibres, an association between meat content and halothane genotype was seen (Wicke *et al.*, 1987). It was concluded that the increase in meat content was partly explained by the increase in fibre area. When comparing the fibre cross-sectional areas of the F_2 animals with the halothane-gene-free Swedish Yorkshire pigs in the study by Karlsson *et al.* (1993), areas were significantly smaller in the latter group (data not shown).

As the histochemical methods are very time consuming, the number of animals included in the study was small, and the observed significances should therefore be interpreted with caution and confirmed by additional data. An overall significance test (Fisher, 1954) was performed by combining the probability values for the mean area of the three fibre types when comparing the ATP1B1 genotypes. The overall probability value for BD and LD were P=0.005 (χ^2=19.1, 6df) and P=0.18 (χ^2=6.1, 6df) respectively. The overall probability value for fibre areas between halothane genotypes was P=0.05 (χ^2=13.9, 6df) in BF and P=0.50 (χ^2=3.3, 6df) in LD. These calculations, and the results presented above concerning comparison between the F_2 animals with halothane-gene-free pigs, may give proof of that the ATP1B1 locus, or a locus linked to ATP1B1, has a superior effect on the muscle fibre cross-sectional areas, compared with the effect of the halothane gene.

CONCLUSIONS

It can be concluded that the genetic marker for the Na^+/K^+-ATPase-gene (ATP1B1) has an influence on the size of the cross-sectional muscle fibre area and muscle metabolism. The results indicate that the ATP1B1 locus, or more likely, a locus linked to ATP1B1, explains a part of the genetic difference in these traits between the wild boar and the domestic pig.

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Table 1. Least squares means ± SE for muscle characteristics and levels of significance[a] between the two ATP1B1 (Ka^+K^+-ATPase)-genotypes.

	m.longissimus dorsi				
	Wild		Domestic		Prob.
	Mean	SE	Mean	SE	
Number	12		8		
Fibre type, %					
Type I	7.9	1.18	9.7	1.50	0.66
Type IIA	6.5	1.10	4.2	1.40	0.22
Type IIB	84.9	1.12	85.5	1.43	0.76
Fibre area, μm^2					
Type I	4727	457	3810	580	0.24
Type IIA	3368	359	2987	483	0.54
Type IIB	6051	663	5034	841	0.36
Glycogen depleted fibre, %					
Type I	24.7	10.0	37.2	13.9	13.9
Type IIA	52.7	10.6	77.4	15.6	15.6
Type IIB	27.8	8.6	39.7	12.3	12.3
Enzyme activity, mmol*kg dry $wt^{-1}min^{-1}$					
CS	8.1	0.67	8.5	0.86	0.68
HAD	15.8	1.18	14.3	1.49	0.44
LDH	3207	141	3145	179	0.79
Glycogen, mmol/kg dry wt	10814		8317		0.80

Table 1 (cont). Least squares means ± SE for muscle characteristics and levels of significance[a] between the two ATP1B1 (Ka^+K^+-ATPase)-genotypes.

	m.biceps femoris		
	Wild	Domestic	Prob.
Number	12	8	
Fibre type, %			
Type I	11.2 2.04	12.2 2.28	0.76
Type IIA	9.3 1.09	7.6 1.22	0.32
Type IIB	79.2 2.68	80.2 3.00	0.82
Fibre area, μm^2			
Type I	3509 152	2779 282	0.08
Type IIA	3106 215	2651 241	0.18
Type IIB	4827 230	3676 258	0.005
Glycogen depleted fibre, %			
Type I	88.2 8.6	85.9 9.7	0.86
Type IIA	88.0 7.9	84.2 8.8	0.76
Type IIB	57.2 7.9	57.0 8.8	0.98
Enzyme activity, mmol*kg dry $wt^{-1}min^{-1}$			
CS	19.3 1.38	17.2 1.70	0.37
HAD	15.7 0.77	13.5 0.95	0.10
LDH	2814 130	3061 160	0.25
Glycogen, mmol/kg dry wt	192 40	209 53	0.81