IN VIVO MUSCLE GLYCOGEN AND PHOSPHATE METABOLISM IN RELATION TO BODY COMPOSITION IN YOUNG PIGS OF DIFFERENT GENOTYPES STUDIED BY NUCLEAR MAGNETIC RESONANCE TECHNIQUES

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SUMMARY

Three ryanodine receptor 1 gene variants (*NN*: homozygous stress stable, *Nn*: heterozygous and *nn*: homozygous stress susceptible) served as a model for stress susceptibility with 96 pigs in each study originating from 6 breeding lines. Their age varied between 4 and 12 weeks and the body weight between 6 and 20 kg. Pig breeding programs which use only the normal genotype (*NN*) will need to apply supplementary selection criteria to reach further improvements in the constitution of the animals and finally in the carcass and meat quality. ^{13}C and ^{31}P nuclear magnetic resonance spectroscopy were applied noninvasively *in vivo* and in a few pigs also *post mortem* to study the metabolic processes in the biceps femoris muscle after halothane exposure. In contrast to no visible effects of the halothane challenge test, the heterozygous defective allele carriers showed a reduction in the levels of glycogen (57 %), phosphocreatine (44 %), ATP (10.5 %) and muscle tissue pH (0.28) coupled with an increase in inorganic phosphate (355 %) and body temperature (1.71 °C). Overall, these changes were intermediate compared to the dramatic response in the homozygous *nn* genotype and to the very slow processes in *NN*. Results of ^{1}H magnetic resonance imaging provided the evidence that the defective allele carriers are leaner than normal pigs already at approximately 11 kg live weight. However, fatter pigs are not necessarily more stress stable.

Keywords: Swine, muscle metabolism, body composition, nuclear magnetic resonance, ryanodine receptor.

INTRODUCTION

Since Fujii et al. (1991) found an association between susceptibility to Malignant Hyperthermia and the alteration of C1843 to T1843 in the DNA encoding the Ca^{2+} release channel of skeletal muscle sarcoplasmic reticulum (ryanodine receptor 1 -- RYR1), the RYR1 gene test has been used intensively in numerous pig breeding programs. As dam lines (and to some extent sire lines) are being ultimately cleared from all defective allele carriers, it will be necessary to use supplementary selection criteria based on the thorough knowledge of metabolic processes to achieve further genetic improvements. *Nuclear Magnetic Resonance* (NMR) techniques like spectroscopy and imaging are very powerful tools to manifest such selection criteria noninvasively and continuously both *in vivo* and *post mortem*. Previous *in vivo* ^{31}P NMR studies established that especially homozygous stress susceptible pigs respond with faster phosphocreatine (PCr) decay, faster declining pH level (higher glycogen depletion), and a simultaneous increase of inorganic phosphate (Pi) combined with an increased adenosine triphosphate (ATP) depletion to muscle stressors. Controversial

inferences were made for the metabolism at rest and metabolic response of heterozygous pigs in comparison with the normal and homozygous stress susceptible genotype, which were mainly caused by confounding RYR1*line effects (Geers *et al.* 1992a,b, 1996; Janzen *et al.* 1994; Kohn 1997).

MATERIALS AND METHODS

Three associated NMR studies were performed with a Varian 4.7 T 33 cm horizontal bore magnet. The RYR1 genotypes (*NN*, *Nn*, *nn*) of 96 pigs in each study -- metabolism or body composition (Table 1) -- were identified by using a polymerase chain reaction technique (Brenig and Brem 1992). They originated from 6 different breeding lines (Table 2) with an age between 4 and 12 weeks and a body weight between 6 and 20 kg. Food was withheld for 18 hours prior to the experiment(s). Ten minutes prior to every spectroscopy experiment, the pigs were sedated and positioned in a cradle. ^{31}P spectra were collected from the M. biceps femoris every minute as described by Scholz et al. (1995). ^{13}C spectra were acquired every 273 seconds with an acquisition time of 0.026 s for each free induction decay, a recovering time of 0.158 s and 1480 pulse repetitions at 50.295 MHz (spectral width = 20000 Hz). Broad band proton noise decoupling was activated only during the acquisition time to avoid the development of the nuclear Overhauser effect. Spectra acquisition was always started 10 minutes (20 minutes during ^{13}C) before halothane administration characterizing the metabolism under resting conditions and continued for 60 minutes (at least for 30 minutes during ^{13}C) after halothane administration (3 Volume% per 3 Liters O_2/minute). Halothane administration was stopped immediately, before reaching the time limit of 60 minutes, after observing a significant drop in PCr or glycogen. *In vivo* and for a few pigs *post mortem* changes in ATP, Pi, PCr, pH values (^{31}P), glycogen (^{13}C) and in the body temperature were monitored during the experiments. ^{1}H magnetic resonance imaging (MRI) was performed at 200.23 Mhz. A Spin-Echo technique generated 5 continuous axial images with a slice thickness of 0.49 cm between the 14th and 12th vertebra. The volume of both longissimus dorsi muscles and of the overlying fat was determined by means of the software ANALYZE 7.01 (Mayo Foundation) or OPTIMAS 5.0 (BioScan Inc.).

A General Linear Model (SAS 6.12) with fixed effects RYR1 genotype, line, gender, final status (*in vivo* or *post mortem*), RYR1*final status interaction and covariates; age and body weight, was applied for the analysis of the metabolic response. Final status was not used in the models for the metabolite levels at rest and for the image data. Only significant effects were kept in the models for each trait to calculate the least squares means and standard errors of estimation (LSM±SE). The time of halothane exposure did not affect the response intensity.

RESULTS AND DISCUSSION

All homozygous defective allele carriers and some of the heterozygous pigs showed dramatic changes in their muscle energy metabolites after halothane exposure, while the concentration of muscle energy metabolites altered very slowly in the less muscled *NN* genotype (Table 1). As described by Scholz et al. (1995), the *in vivo* metabolic processes in the *Nn* genotype tended to be more similar to the *NN* genotype. Differences among all three genotypes became more evident *post mortem* with *Nn* being intermediate. The same tendency appeared for the

glycogen depletion (%) during the ^{13}C NMR study, though the *Nn* genotype showed an even higher response than *nn* with LSM (±SE) *post mortem* of 20.50±12.71 in the *NN* genotype, 77.68±7.20 in *Nn*, and 53.22±7.52 in *nn*. No differences among the RYR1 genotypes were detected in the *in vivo* muscle tissue pH (≈7.06) under resting conditions, but *Nn* had a lower body temperature than *NN* and *nn*. In contrast to the results of Kohn (1997), *nn* pigs showed already at rest a lower phosphorilation potential (PCr/Pi-ratio) than *NN* and *Nn* pigs, which was caused by a slightly higher Pi starting level (not shown in the table). Similar observations were made during the ^{13}C experiment with a significantly higher glycogen starting level in *NN* and *Nn* pigs compared to *nn*. In contrast to these results, Lindner (1991) found no significant differences in the glycogen level at rest between stress stable and stress susceptible pigs using *in vivo* biopsy samples. Behavioral differences observed in a few pigs prior to anesthesia may have resulted in different 'starting values' among the genotypes.

Table 1. Metabolic response, body temperature change, time of halothane exposure and body composition in pigs of different ryanodine receptor 1 genotypes

	NN	Nn	nn
PCr/Pi ratio start	15.53±.69[a]#	15.21±.52[a]	13.35±.79[b]
Phosphocreatine (PCr) drop (%)	32.11±5.27[a]	44.01±4.17[b]	72.12±5.63[c]
inorganic Phosphate (Pi) increase (%)	394±39[a]	355±33[ab]	291±43[b]
pH minimum	6.908±.041[a]	6.769±.035[b]	6.563±.046[c]
ATP change (%)	+4.02±4.24[a]	-10.53±3.63[b]	-37.74±4.73[c]
total n (n -- *post mortem*)	24 (3)	29 (4)	16 (3)
Glycogen start (µmol/g)	74.12±5.91[a]	76.11±5.15[a]	57.12±4.35[b]
Glycogen drop (%)	20.88±8.45[a]	56.71±5.11[b]	46.76±3.51[b]
total n (n -- *post mortem*)	7 (1)	10 (2)	10 (3)
Body temperature start (°C)	39.38±.14[a]	38.96±.13[b]	39.25±.15[ab]
Body temperature change (°C)	-.71±.34[a]	+1.70±.29[b]	+2.82±.33[c]
Halothane (minutes)	44.16±2.90[a]	38.43±2.59[a]	6.57±3.02[b]
total n ^{13}C+^{31}P (n -- *post mortem*)	31 (4)	39 (6)	26 (6)
Volume Muscle l.d. (cm^3)	40.78±1.64[a]	41.24±1.53[a]	46.53±1.91[c]
Fat volume (cm^3)	13.19±.69	12.08±.65	13.58±.82
n	31	42	23

#) Least Squares Means (±SE) with different superscripts are significantly different ($p<0.05$).

Though female pigs (n=10) had a significant higher glycogen starting level (p=0.0128) than male pigs (n=17) with LSM of 76.83 µmol/g and 61.40 µmol/g, no significant differences occurred in the relative glycogen depletion. In contrast, differences were significant both in the glycogen and in the PCr depletion comparing different lines (Table 2). Surprisingly, the most obese line(s) originating mainly from Spotted Poland China responded more dramatically to halothane exposure than the leaner lines with a higher degree of Hampshire (RN locus effect !), Poland China•Landrace, Landrace or Duroc origin. This implies, that a low body fat content does not necessarily coincide with high stress susceptibility, while a high

body fat content is not basically associated with stress stability. However, the effect of the RYR1 genotype is responsible for about 'only' 20 % of the variation in the stress (PCr) response and accounts for a relatively small portion of the variation in the body composition traits (3-17%), leaving 'substantial' variation for further genetic improvements.

Table 2. Metabolic response and body composition in pigs of different breeding lines

	≥50 % Duroc (D)	≥50 % Hampshire (H)	Landrace (L)	Poland China (PCh)•L	≥50% Spotted PCh (S)
PCr/Pi ratio▼	15.50±.49[a]#	15.53±.87[a]	10.78±.90[b]	14.95±1.00[a]	16.71±1.24[a]
PCr drop (%)	51.07±3.96[ac]	38.94±6.28[b]	45.74±6.35[ab]	44.41±7.18[ab]	66.90±8.91[c]
Pi increase (%)	323±26[a]	352±42[a]	275±43[a]	273±48[a]	510±59[b]
pH start▼	7.05±.01[a]	7.05±.01[a]	7.04±.01[a]	7.08±.02[ab]	7.09±.02[b]
pH minimum	6.76±.03[ab]	6.71±.04[a]	6.74±.04[a]	6.83±.05[b]	6.71±.06[ab]
ATP drop (%)	11.59±2.86[a]	10.86±4.58[a]	7.28±4.65[a]	8.39±5.26[a]	35.64±6.46[b]
n	29	12	14	9	5
Fat/Muscle ratio	.33±.02[acd]	.27±.03[b]	.29±.04[bd]	.33±.03[abc]	.42±.05[ac]
n	34	16	7	28	5
Glycogen▼(µmol/g)	66.48±5.05	69.72±5.15	67.63±5.57*		72.63±10.43
Glycogen drop (%)	40.31±4.73[b]	27.28±8.87[a]	42.48±4.56[b]		55.74±12.58[b]
n	9	7	9 (•.28±.05[ab]; n=6)		2

#) Least Squares Means (±SE) with different superscripts are significantly different ($p<0.05$).
▼) at rest. *) 25% D, 25% H, 25% L, 12,5% S, 12,5% Yorkshire. •) Fat/Muscle ratio.

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