Effect of foetus weight at day 70 of gestation on muscle fiber number, myosin heavy chain isoform expression and proteomic pattern in three porcine muscles. A preliminary study

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Introduction

Due to a high prolificacy, natural intra-uterine growth retardation is a common feature in the pig species, inducing a reduction in mean birth weight (BtW) and an increase in its variability (Quiniou et al., 2002). However, the mechanisms involved are not yet clearly understood. It has been shown that, compared to their heavier siblings, low BtW pigs have impaired growth, fatter carcasses (Bee, 2004) and a lower proportion of skeletal muscle at slaughter, partly due to a reduction in the total number of fibers (TNF) (Rehfeldt and Kuhn, 2006). Myogenesis is a biphasic phenomenon in pigs. Thus, a primary generation of myotubes, called primary fibers (P), is formed from d 35 to 55 of gestation, followed by a second generation, called secondary fibers (S), which develops between d 55 and 90 of gestation. These S appear around each P, using them as a scaffold. In the pig, the TNF is considered to be fixed at d 90 of gestation (Lefaucheur, 2001). The aims of this preliminary study were the following: 1) establish, which techniques can be used to determine the TNF and the S/P ratio, 2) determine myosin heavy chain (MyHC) polymorphism, and 3) characterize the skeletal muscle proteome in the porcine muscle. Furthermore, we were interested to study the relationship between TNF, S/P ratio, expression of MyHC isoforms and muscle proteome and fetal weight (FW) in pig fetuses at d 70 of gestation, a stage where all P are created and the S are formed.

Material und Methods

One heterogeneous litter (n = 8 fetuses; average litter weight ± s.e. = 232 ± 47 g) was selected from a cross-bred (Large White × Landrace) sow mated with a Large White boar. All eight fetuses were selected and classified into three FW groups: Low (L): < 190 g, High (H): > 270 g, and Medium (M): 210-250 g. Three muscles, semitendinosus (ST), longissimus dorsi (LD) and rhomboideus (RH) were collected and frozen in isopentane cooled in liquid nitrogen for later histological and 1D and 2D gel analysis. Histological analyses were carried out in 10 µm-thick muscle transverse serial sections. Immunohistochemistry using anti-Slow(-I) and anti-Fast(-II)-Fetal MyHC monoclonal

antibodies (1:20; Novocastra, Newcastle. UK), was carried out according to the procedure described by Lefaucheur et al. (2002). The anti-Slow(-I) MyHC antibody allowed differentiating the dark deep (STD) from the light superficial portions (STL) of the ST. The actomyosin ATPase staining, after pre-incubation at pH 4.35, was used to determine the TNF in the RH and ST as previously described by Lefaucheur et al. (2002). Skeletal muscle proteome and MyHC polymorphism were determined in the LD using 2D (Bouley et al., 2004) and 1D gel electrophoresis (Talmadge and Roy, 1993), respectively.

Results and Discussion

Results obtained using the anti-Slow(-I) and anti-Fast(-II)-Fetal MyHC monoclonal antibodies revealed that STD and STL express different MyHC isoforms at d 70 of gestation in pig fetuses. The MyHC anti-Slow(-I) antibody marked the P more distinct in the STD than in the STL. Contrarily, the P of the STD were weakly marked with the MyHC anti-Fast(-II)-Fetal antibody, but strongly marked in the STL (Figure 1). It is known that the anti-Fast(-II)-Fetal MyHC antibody reacts with both, the fetal as well as the fast type II MyHC isoforms. However, we found that the fast type II MyHC isoforms was not expressed. This later finding is based on results obtained by using 1D SDS-PAGE, which revealed that the Fast(-II) MyHC isoform was not yet expressed in the LD at d 70 of gestation. These results confirm that at this stage of development the STD is more mature than the STL because during myogenesis the Slow(-I) MyHC isoform appears after the Fetal MyHC isoform (Lefaucheur, 2001).

As expected, the average FW among the FW classes differed ($P < 0.01$; Table 1). Although the number of animals was limited, the results suggest that the H-FW group exhibited the highest ($P < 0.05$) number of both, P and S in the STL and of S ($P < 0.05$) in the STD (Table 1). As a result, fetuses in the H-FW class had higher TNF in the ST compared to fetuses of the M- and L-FW classes. This increase was primarily due to an increase in the number of P while the S/P ratio did not differ ($P > 0.05$) between FW classes. These findings confirm that the number of P is a major determinant of TNF. In the RH the number of P tended ($P = 0.07$) to be greater in the H–FW than the M- and L-FW group, but contrary to the ST, the differences were not large enough to affect the TNF.

In the LD, fifteen proteins were expressed differently between the three FW-groups. In particular, transferrin content was greater ($P < 0.01$) in the LD of fetuses of the H- than the M- and L-FW groups. Transferrin is a blood plasma protein for iron ion delivery. Although iron bound to transferrin is less than 0.1% of the total body iron, dynamically it is the most important iron pool,

with the highest turnover rate. We report a positive ($r = 0.82$; $P < 0.01$, n=8) correlation between the transferrin content in the LD and the TNF in the ST and RH, which could suggest that fetuses of the H-FW class had a more developed muscle vascularisation than those of the M- and L-FW classes. This could then explain the higher number of P and consequently the higher TNF in the ST.

Table 1. Effect of fetal weight on muscle fiber number of semitendinosus muscle (ST) and rhomboideus muscle (RH) and on the transferrin content of the longissimus dorsi muscle (LD).

	Fetal weight[1]			
Trait	**L-FW**	**M-FW**	**H-FW**	**SE**
Number of fetuses	3	3	2	
Average FW classes	170[a]	231[b]	287[c]	10.5
Muscle ST				
Whole muscle				
TNF[2]	141218[a]	159469[a]	221659[b]	9490
Number of primary fibers (P)	10934[x]	13134[xy]	17109[y]	1497
Number of secondary fibers (S)	130284[a]	146335[a]	204550[b]	8577
S / P ratio	11.3	11.9	12.4	1.4
Dark portion				
TNF[2]	71482[a]	79325[a]	115565[b]	8281
Number of primary fibers (P)	5856	7315	9536	1239
Number of secondary fibers (S)	65626[a]	72009[a]	106029[b]	7276
S / P ratio	10.2	11.2	12	1.66
Light portion				
TNF[2]	69736[a]	80144[a]	106093[b]	4956
Number of primary fibers (P)	5078[a]	5818[ab]	7572[b]	513
Number of secondary fibers (S)	64657[a]	74326[a]	98521[b]	4736
S / P ratio	12.9	12.9	13	1.2
Muscle RH				
TNF	34852	41020	68938	11886
Number of primary fibers (P)	1945[x]	2260[x]	3531[y]	367
Number of secondary fibers (S)	32907	38760	65407	11532
S / P ratio	17.0	17.3	17.8	2.1
Muscle LD				
Transferrin, AU[3]	2.34[a]	3.21[b]	4.92[c]	0.33

[a,b,c] Within a row, least squares means without a common superscript differ ($P \leq 0.05$)
[x,y] Within a row, least squares means without a common superscript differ ($P \leq 0.10$)
[1] L-FW = low foetus weight, M-FW = medium foetus weight, and H-FW = High foetus weight
[2] TNF: total number of fibers
[3] AU: Arbitrary unit

Conclusions

These results suggest that the number of P is an important factor to explain the higher TNF in the heaviest fetuses. The differences in transferrin expression found might be related to muscle vascularisation, which then could explain differences in myogenesis.

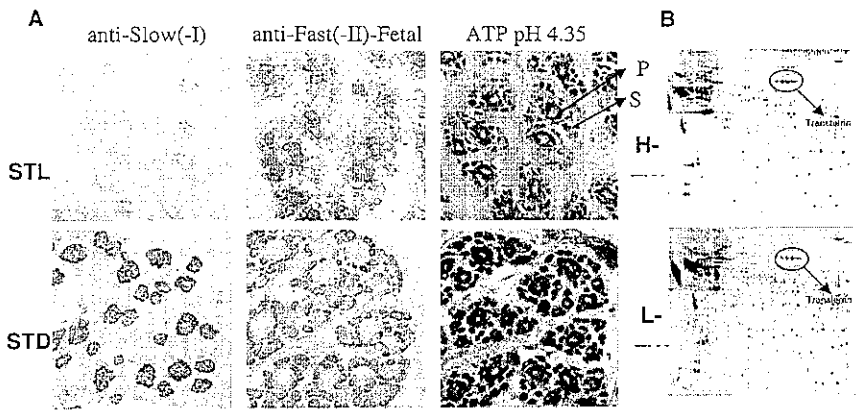

Figure 1. A: Primary (P) and secondary (S) myotubes on serial sections in the light (STL) and dark (STD) portion of the semitendinosus muscle at d 70 of gestation in pigs, using anti-Slow(-I) and anti-Fast(-II)-Fetal MyHC monoclonal antibodies and actomyosin ATPase staining, after preincubation at pH 4.35). B: Skeletal muscle proteome of the longissimus dorsi determined in fetuses of the high (H-) and low (L-) foetus weight (FW) groups.

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