Consequences of pig domestication for skeletal muscle growth and cellularity

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Abstract

This study was conducted to investigate the impact of domestication of the pig on various cellular properties of skeletal muscle. For this purpose, samples of \textit{semitendinosus} (ST), \textit{psosas major} (PM), and \textit{longissimus} (LD) muscles of 24 European wild boars (WB; \textit{Sus scrofa scrofa}) and of 28 domestic pigs (\textit{Sus scrofa domestica}) of German Landrace (DP) were taken at birth, and at 7 and 20 weeks of age and subjected to histological, biochemical, and/or cell culture studies. At birth, DP muscles exhibited lower numbers of myofibers and were less mature, as seen by DNA, RNA, and protein composition and lower proportions of type I fibers. Satellite cell cultures from neonatal ST and LD muscles of WB grew more intensely than that of DP. Additional fiber formation shortly after birth occurred at a higher rate in ST, but not in PM, of DP compared with WB. During postnatal growth, the higher gain in muscle mass of DP resulted mainly from accelerated myofiber hypertrophy and increased protein accretion at the level of transcription. DP muscles exhibited higher proportions of fast-twitch (IIb) and/or white glycolytic fibers than WB. Conclusively, domestication of the pig substantially changed the ontogenic development and the contractile and metabolic properties of skeletal muscle.

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1. Introduction

The recent European domestic pig is the result of a long-lasting process of domestication, which includes the exposure of its ancestors, the European wild boar, both to artificial selection and substantial changes in the environment. Considering the recent wild-type pig to be similar to the ancestors of our domestic pig, their comparison may provide insight into the effects of domestication on various economically important traits of pig production. But, in addition, differences in causal sub-traits that determine growth performance, carcass and meat quality can be used as first information of their genetic control. Subsequently, crossing experiments between wild pigs and domestic pigs may serve to detect quantitative trait loci (QTL) by linkage analyses (Andersson et al., 1997; Andersson-Eklund et al., 1998; Knott et al., 1998; Nii et al., 2005). Finally, these QTL allow to derive positional candidate genes that with high probability are involved into the regulation of these traits.

Growth, carcass composition, and meat quality are important trait complexes in pig production, but they are known to depend on a variety of underlying factors. In
the past, pig performance, mainly represented by litter size, daily gain, feed conversion efficiency and lean meat content, has been substantially improved by selection. However, adverse effects in stress resistance and meat quality have accompanied these advances. Lean accretion and meat quality are significantly determined by the morphological and physiological properties of the muscle fibers as the major constituents of skeletal muscle (e.g. Larzul et al., 1997; Henckel et al., 1997; Fiedler et al., 1999, 2004), and, in part, these properties may explain the antagonism between these important traits (Rehfeldt et al., 2000; Rehfeldt and Kuhn, 2006). Moreover, looking at cellular and subcellular components and their expression in skeletal muscle might bring us a step closer to the genes that regulate the complex phenotype (Wimmers et al., 2006).

There are some studies that have compared muscle characteristics of wild-type and domestic pigs, which provided valuable information. They revealed that domestication has been associated with large increases in the size of the muscle fibers and with changes in fiber type composition towards the fast-twitch glycolytic property (Bader, 1983; Solomon and West, 1985; Weiler et al., 1995; Fiedler et al., 1998; Müller et al., 2002). The aim of this study was to extend this comparison to a series of additional muscle characteristics, to neonatal piglets and to myogenic satellite cell cultures to further elucidate the influence of domestication on skeletal muscle development. This information will be useful for genetic improvement of the domestic pig.

2. Materials and methods

2.1. Animals and samples

Domestic pigs (Sus scrofa domesticus) of the German Landrace (n = 28) and European wild boars (Sus scrofa scrofa; n = 24), further termed as DP and WB, respectively, were used in this study. Sows and their offspring were kept in group housing in the experimental station of the Institute for Animal Breeding Mariensee, Neustadt, Germany. DP were fed ad libitum with typical commercial diets according to recommendations for weanling and growing pigs from the experimental station of the Research Institute of the Biology of Farm Animals, Dummerstorf, Germany.

Pigs were slaughtered at three stages of age, i.e. one day after birth, and 7 and 20 weeks of age. Carcasses were dissected and samples from semitendinosus (ST), psoas major (PM) and longissimus thoracis et lumborum (LD), muscles were collected from newborn piglets and/or from growing pigs at 7 and 20 weeks of age within 15–20 min post mortem. Weight, circumference and length of ST and PM muscles were recorded. Muscle cross sectional area (MCSA) was estimated from the circumference of the muscle mid belly.

2.2. Histology, histochemistry, microscopy

From newborn piglets, pieces of the left-side ST muscle mid belly were mounted on cork-chucks and snap-frozen in isopentane cooled in liquid nitrogen. Whole muscle serial transverse sections of 10 and 16 μm were cut at −20 °C in a cryostat (Reichert-Jung, Leica, Nussloch, Germany) and stained for myosin ATPase (EC 3.6.1.32) after acid preincubation at pH 4.2 according to Guth and Samaha (1970) and with eosin (Romeis, 1989), respectively. To determine the total muscle fiber number per cross section in neonatal samples of ST muscle, microscopic images of randomly selected areas of eosin-stained sections were projected on a table and the fibers were counted over an area of ca. 10% of the total MCSA by a pen-counter and extrapolated to the total MCSA.

In pigs of 7 and 20 weeks of age, muscle fiber characteristics were measured in LD, ST, and PM muscle. From left ST muscle one sample each was collected from the dark (deep) and bright (superficial) portion of the mid belly, and one sample was taken from the central part of the left PM muscle mid belly. Samples were mounted on cork-chucks and snap-frozen in liquid nitrogen. Serial sections were cut at 10 μm and stained for cytoplasm and nuclei by haematoxylin/eosin (Romeis, 1989) or for alkaline phosphatase (Gomori, 1952, described by Spannhof, 1967) to visualize capillaries. Another two sections were exposed to the reaction for NADH-tetrazolium reductase (NADH-TR; Novikoff et al., 1961) or acid-preincubated ATPase at pH 4.2 (Guth and Samaha, 1970), which enables to classify slow-twitch type I, fast-twitch type IIa and fast-twitch type IIb muscle fibers or red intermediate and white muscle fibers, respectively. Fiber type distribution and fiber cross sectional area (FCSA) and the myonuclear/capillary distribution were determined on 400–500 muscle fibers (50% in the dark, 50% in the bright region for ST) by image analysis (AMBA, IBSB, Berlin, Germany). The number of fibers per unit area was used to estimate the total number of fibers by multiplication with the MCSA in ST and PM muscles.

2.3. Biochemical analyses of samples

Samples from LD, ST, and PM muscles were analyzed for DNA, RNA, and protein. DNA was measured fluorometrically by a Cytofluor 2500 plate reader (Perceptive Biosystems, Wiesbaden, Germany) against a standard of calf thymus DNA (Serva, Heidelberg, Germany) after using Hoechst 33258
(Sigma-Aldrich, Deisenhofen, Germany) according to Rehfeldt and Walther (1997). Protein concentration was determined with bicinechonic acid (Sigma-Aldrich) (Smith et al., 1985), and RNA was quantified according to Munro and Fleck (1966).

2.4. Satellite cell culture

The right ST and LD muscles from the newborn piglets (from 2 to 5 per WB or DP) were excised and trimmed of visible connective tissue. Digestion of tissue to release myogenic cells by breed and muscle was carried out according to a modification of a protocol as described previously for mouse muscle (Rehfeldt et al., 2002), which in turn is based on a protocol given by Harper et al. (1987). The satellite cells from ST and LD (ST–SC; LD–SC) were then enriched from the primary myogenic cell suspension by using a Percoll (Sigma-Aldrich, Deisenhofen, Germany) gradient (90, 40, 25% in PBS (137 mM NaCl, 2.7 mM KCl, 3.2 mM Na2HPO4; pH 7.4)) according to an adapted protocol given by Roe et al. (1989). Aliquots of the cells were frozen in liquid nitrogen until required using DMEM (Dulbecco’s minimum essential medium) containing 20% fetal bovine serum (FBS, lot 40F6932X), both obtained from Invitrogen, Karlsruhe, Germany, and 10% dimethyl sulfoxide (DMSO; Serva). Prior to starting various experiments, cells of the four lines obtained were thawed again and multiplied over four passages in DMEM supplemented with 0.02 M glutamine (Serva, Heidelberg, Germany), 100 IU/ml penicillin, 100 μg/ml streptomycin, 2.5 μg/ml fungizone (Sigma-Aldrich) and 20% FBS (lot 40F4498K).

An aliquot from the 4th-passage cells has been taken to determine the percentages of myoblasts by immunostaining using a mouse monoclonal antibody against desmin from pig stomach (mAb DE-U-10, ascites fluid; D 1033 Sigma-Aldrich) according to a modification of a protocol given by Yablonka-Reuveni et al. (1999), which is described in detail by Kalbe et al. (2002). Prior to starting various experiments, the cells from the four lines obtained were thawed again and multiplied over four passages in DMEM supplemented with 0.02 M glutamine (Serva, Heidelberg, Germany), 100 IU/ml penicillin, 100 μg/ml streptomycin, 2.5 μg/ml fungizone (Sigma-Aldrich) and 20% FBS (lot 40F4498K).

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All data were subjected to analyses of variance, using the mixed or GLM classification models of SAS® (SAS Inst. Inc., Cary, NC, USA). Data shown in tables and graphs are least squares means ± SE. Individual differences between least squares means were tested by Student’s t-test. Significance of differences was concluded at P<0.05.

Genotype, age, sex and their interactions were included as fixed factors in the ANOVA of the data on weights and tissue analyses. In cell culture experiments on growth kinetics, fixed factors were cell line, day of cultivation, row and column of the plate. In addition, the plate was included as a random factor within day of cultivation. In DNA-synthesis experiments, fixed factors were experiment, cell line and FBS concentration.

3. Results

3.1. Body and muscle weights

The comparison of DP with WB at different stages of postnatal life, such as birth, 7 and 20 weeks of age, shows that DPs were already heavier at birth (136% of WB; 1.31±0.05 vs. 0.96±0.07 kg) and subsequently also
grew faster than WBs (Fig. 1). At 7 weeks of age the body weight of DP was 11.63±0.60 kg compared with 4.69±0.70 kg in WP (248%) and at 20 weeks of age it was nearly four times greater than that of WBs (78.25±3.92 vs. 22.07±1.88 kg). Following the development of ST muscle weight, it was already clearly heavier in DP than WB at birth (165%), and the difference was increasing up to 453% with age (Fig. 2). The relative differences in PM muscle weight were smaller and finally DPs reached 293% of WBs. Whereas the differences in length development for ST and PM muscles were almost identical between DP and WB (from ca. 120% at birth to ca.150% at week 20), the differences in the circumference and MCSA were much greater for ST (up to 492%) than for PM (up to 65%) (data not shown). Thus, the PM muscle appeared proportionally, whereas the ST appeared disproportionally enlarged in DPs compared with WBs.

3.2. Myofiber structural properties

3.2.1. Total muscle fiber number

The total number of muscle fibers per cross section increased both in ST and PM muscle between birth and 7 weeks of age, but remained unchanged afterwards (Fig. 3). At birth, the number of fibers was lower both in ST (81%) and PM (70%) muscle of DP compared to WB. This was associated with lower numbers of primary muscle fibers in ST (P<0.13) and PM (P<0.05) (Fig. 4) without differences in the percentage of primary fibers (data not shown). In addition, the proportion of type I fibers tended to be lower in ST muscle of DP compared to WB (13.0 vs. 9.2%; P=0.07) indicating a delayed conversion of type II fibers adjacent to the primary fibers to type I in DP. In PM muscle, type I fiber percentage was also numerically lower (10.3 vs. 13.1%) but without statistical significance (P=0.33). The total number of muscle fibers remained lower in PM muscle of DP at 7 and 20 weeks of age. In contrast, the

Fig. 2. Weight development of semitendinosus and psoas major muscles from birth to 20 weeks of age. Data presented are least squares means±SE (*P<0.05).

Fig. 3. Development of the apparent total number of myofibers in two muscles of wild boars and domestic pigs from birth to 20 weeks of age. Data presented are least squares means±SE (*P<0.05).

Fig. 4. Proportions of primary fibers (A) and type I fibers (B) in cross sections from semitendinosus (ST) and psoas major (PM) muscles of newborn wild-type and domestic pigs. Least squares means±SE are represented as columns and error bars, respectively (*P<0.05; †P=0.07; ‡P=0.13).
3.2.2. Myofiber size

Measurements of the myofiber cross sectional areas (FCSA) at 7 and 20 weeks of age (Table 1) revealed that the fibers were significantly larger in DP compared to WB in all three muscles examined ($P<0.05$). The FCSA was almost doubled with the largest differences seen in ST muscle (up to 268% of WB). At 20 weeks of age the largest fibers were exhibited in ST muscle compared to PM and LD muscle both in WB and DP. Looking at the individual fiber types, the fibers of each type were larger in DP than in WB muscles. It is worth noting that the contractile fiber types (I, IIa, IIb) were almost equal in size at 7 weeks of age, whereas at 20 weeks of age the type IIb fibers were the largest in all muscles both in DP and WP.

3.2.3. Fiber type distribution

The distribution of fiber types has been determined separately according to the classification for contractile (I, IIa, IIb) or metabolic (red, intermediate, white) properties (Fig. 5A and B). The differences between DP and WB were dependent on the muscle examined. In the LD muscle both at 7 and at 20 weeks of age significantly higher percentages of IIb fibers and lower percentages of IIa fibers were observed. At week 20, also more type I fibers were found in DP as compared to WB ($P<0.05$). With respect to metabolic fiber types, the LD of DP displayed significantly more white, glycolytic fibers and less fibers of the intermediate (7 weeks) or red type (20 weeks) than the LD of WB. It is worth noting that in LD the percentages of white fibers were almost identical with that of type IIb fibers, but the proportions of red and intermediate fibers were inconsistent with the proportions of type I and IIa fibers, respectively. All type I fibers were highly oxidative (red), but some of the IIa fibers, too.

In summary, a shift to the fast-twitch glycolytic property is apparent in skeletal muscle of domestic pigs, and this is clearly exhibited at 20 weeks of age.

Table 1
Fiber cross sectional area (FCSA) in $\mu m^2$ of contractile fiber types I, IIa, IIb determined by myosin ATPase histochemistry in three muscles from wild-type (WB) and domestic pigs (DP) slaughtered at different stages of age

<table>
<thead>
<tr>
<th>Item</th>
<th>Weeks of age</th>
<th>WB</th>
<th>DP</th>
<th>$P \leq$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>7</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No. of pigs</td>
<td>8</td>
<td>11</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ST</td>
<td>Average</td>
<td>407±43</td>
<td>972±39</td>
<td>0.0001</td>
</tr>
<tr>
<td></td>
<td>Type I</td>
<td>449±104</td>
<td>948±93</td>
<td>0.01</td>
</tr>
<tr>
<td></td>
<td>Type IIa</td>
<td>369±41</td>
<td>898±36</td>
<td>0.0001</td>
</tr>
<tr>
<td></td>
<td>Type IIb</td>
<td>418±68</td>
<td>1001±61</td>
<td>0.0001</td>
</tr>
<tr>
<td></td>
<td>Average</td>
<td>328±56</td>
<td>532±50</td>
<td>0.05</td>
</tr>
<tr>
<td></td>
<td>Type I</td>
<td>453±38</td>
<td>559±36</td>
<td>0.06</td>
</tr>
<tr>
<td></td>
<td>Type IIa</td>
<td>305±33</td>
<td>453±31</td>
<td>0.01</td>
</tr>
<tr>
<td></td>
<td>Type IIb</td>
<td>318±71</td>
<td>564±67</td>
<td>0.05</td>
</tr>
<tr>
<td>PM</td>
<td>Average</td>
<td>409±78</td>
<td>674±69</td>
<td>0.05</td>
</tr>
<tr>
<td></td>
<td>Type I</td>
<td>500±62</td>
<td>608±56</td>
<td>0.22</td>
</tr>
<tr>
<td></td>
<td>Type IIa</td>
<td>381±54</td>
<td>492±49</td>
<td>0.15</td>
</tr>
<tr>
<td></td>
<td>Type IIb</td>
<td>431±96</td>
<td>766±86</td>
<td>0.05</td>
</tr>
<tr>
<td>LD</td>
<td>Average</td>
<td>409±78</td>
<td>674±69</td>
<td>0.05</td>
</tr>
<tr>
<td></td>
<td>Type I</td>
<td>500±62</td>
<td>608±56</td>
<td>0.22</td>
</tr>
<tr>
<td></td>
<td>Type IIa</td>
<td>381±54</td>
<td>492±49</td>
<td>0.15</td>
</tr>
<tr>
<td></td>
<td>Type IIb</td>
<td>431±96</td>
<td>766±86</td>
<td>0.05</td>
</tr>
</tbody>
</table>

ST—semitendinosus, PM—psoas major, LD—longissimus thoracis et lumborum.
Data presented are least squares means±SE.

ST total fiber number of DP clearly exceeded that of WB (130–155%) resulting from a much higher postnatal increase in the apparent fiber number between birth and 7 weeks of age.

3.2.2. Myofiber size

Measurements of the myofiber cross sectional areas (FCSA) at 7 and 20 weeks of age (Table 1) revealed that the fibers were significantly larger in DP compared to WB in all three muscles examined ($P<0.05$). The FCSA was almost doubled with the largest differences seen in ST muscle (up to 268% of WB). At 20 weeks of age the largest fibers were exhibited in ST muscle compared to PM and LD muscle both in WB and DP. Looking at the individual fiber types, the fibers of each type were larger in DP than in WB muscles. It is worth noting that the contractile fiber types (I, IIa, IIb) were almost equal in size at 7 weeks of age, whereas at 20 weeks of age the type IIb fibers were the largest in all muscles both in DP and WP.
3.2.4. Distribution of capillaries

Remarkable differences between DP and WB were observed in the muscle fiber area associated with one capillary (Fig. 6). At 7 weeks of age, the average FCSA associated with one capillary was 367%, 393%, and 583% in DP compared with WB in LD, PM, and ST, respectively. The differences were significant for all of the metabolic fiber types, but were largest for the white type (data not shown). Similar differences were apparent at 20 weeks of age. The number of capillaries per myofiber as another indicator of capillary density tended to be lower in ST muscle ($P=0.06$) of DP compared with WB at 7 weeks of age, but showed no differences in LD and PM muscles or at 20 weeks of age (data not shown).

3.2.5. Myonuclear distribution

The differences in the number of myonuclei per fiber and per fiber area between DP and WB show identical directions in all of the three muscles (Fig. 7A,B). The average nuclear number per fiber was not different between DP and WB at 7 weeks of age. At 20 weeks of age, the nuclear number per fiber was higher in DP (1.6–1.8) than in WB (0.9–1.1) with higher values in all

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**Fig. 5.** Proportions of contractile (A; ATPase histochemistry) and metabolic fiber types (B; NADH tetrazolium reductase histochemistry) in cross sections of *semitendinosus* (ST), *psoas major* (PM) and *longissimus* (LD) muscles of wild-type (WB) and domestic pigs (DP) at 7 and 20 weeks of age. Least squares means are represented as columns (SE for metabolic types=1.4–7.8%; SE for contractile types=1.1–5.5%; $*P<0.05$; $P=0.06$).

**Fig. 6.** Capillary density measured as fiber area/capillary in cross sections of *semitendinosus* (ST), *psoas major* (PM) and *longissimus* (LD) muscles of wild-type (WB) and domestic pigs (DP) at 7 and 20 weeks of age. Least squares means±SE are represented as columns and error bars, respectively ($*P≤0.05$; $P=0.06$; $+P=0.13$).
contractile fiber types (data not shown). The nuclear number per muscle fiber area, however, was much lower in the muscles from DP than from WB both at 7 and 20 weeks of age. All differences are statistically significant with the exception of LD at week 7 ($P_{=}0.16$) and PM at week 20 ($P_{=}0.12$). For all fiber types the number of nuclei per mm$^2$ was numerically lower (data not shown).

### 3.2.6. Muscular protein, RNA and DNA

At birth, the muscles from DP displayed lower DNA, RNA, and protein concentrations than muscles from WB as shown for ST and PM muscles (Fig. 8A). At 7

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#### Fig. 7. Myonuclear density measured as the number of nuclei/fiber (A) and nuclei/mm$^2$ fiber area (B), respectively, in cross sections of semitendinosus (ST), psoas major (PM) and longissimus (LD) muscles of wild-type (WB) and domestic pigs (DP) at 7 and 20 weeks of age. Least squares means±SE are represented as columns and error bars, respectively ($^\circ\!P_{=}0.05; ^\circ\!P_{=}0.06; ^\circ\!P_{=}0.10–0.12$).

#### Fig. 8. Protein, DNA, and RNA concentrations (A) and RNA/DNA and DNA/protein ratios (B) of semitendinosus (ST) and psoas major (PM) muscles in wild boars and domestic pigs slaughtered at different stages of age. Data presented are least squares means±SE ($^\circ\!P_{=}0.05; ^\circ\!P_{=}0.06$).
and 20 weeks of age, differences in RNA and protein concentrations between the genotypes were no longer observed. However, muscular DNA concentrations remained significantly lower at 7 weeks of age in DP, but were no longer significantly different from WB at week 20. The changes in these individual components lead to lower RNA/DNA and higher DNA/protein ratios in DP compared to WB at birth, which turned to the opposite at 7 weeks of age (Fig. 8B). At 20 weeks of age these ratios were no longer different between the genotypes, and the protein/RNA ratio (data not shown) was not different at all.

Despite lower DNA concentration the total amount of DNA in ST muscle, calculated via muscle weight, was higher in DPs than in WBs at birth, whereas total RNA and protein were not different (data not shown). In PM muscle, these total amounts were not significantly different between the genotypes, although total RNA and protein tended to be lower in DPs. At 7 weeks of age the total amounts of DNA, RNA, and protein were clearly higher in ST muscle ($P < 0.001$) but only tended to be higher in PM muscle of DP compared with WB. At week 20, the total amounts of all components were substantially higher both in ST and PM muscle of DP compared with WB.

3.3. In vitro growth of muscle satellite cells

3.3.1. Growth kinetics

The growth kinetics of muscle satellite cell cultures derived from two different muscles from domestic and wild-type pigs was analyzed by changes in DNA and protein over 10 days of cultivation in growth-promoting medium.

Regular microscopic evaluation of the plates revealed that the cultures were confluent at days 5–6. No distinct myotube formation could be observed. On day 10 of cultivation, the monolayers of the WB-ST cells had detached from the bottom in many wells, why at this point not all samples could be included into analysis. Analyses of variance revealed clear influences of the factors cell line, day of cultivation and their interactions. As illustrated in Fig. 9, both DNA and protein content increased significantly over time of cultivation ($P < 0.0001$). In all cell lines, after a small increase in DNA content from days 2 to 4 of cultivation, a steep increase occurred from days 4 to 5. Thereafter, phases of stagnation (days 5–7; days 8–9) alternated with phases of proliferation (days 7–8; days 9–10). Protein amount increased approximately linearly from days 3 to 10 and started to plateau for WB-ST cells at the end of cultivation.

The WB cells grew faster than the DP cells. The WB-ST cells showed significantly higher protein and DNA amounts than the DP-ST cells from days 3/4 of cultivation. Likewise, the WB-LD cells grew faster than the DP-LD cells with significant differences being apparent from days 5/6 onward. Within WB, the cells from ST muscle grew faster than those from LD muscle in terms of DNA and protein accumulation. Within DP, the cells from ST accumulated significantly more DNA than LD cells from day 6 onward, but higher protein values were found at day 7 only, and even lower values at days 8–10.

3.3.2. Influence of serum concentration

To study, whether the differences in the growth of WB and DP satellite cell cultures are dependent on the concentration of growth factors in the cellular environment, DNA accumulation and DNA synthesis rate were measured between days 4 and 5 of cultivation using 5, 10, and 20% FBS in growth medium (Fig. 10).

WB cells accumulated more DNA until day 4 of cultivation (Fig. 10A), which confirms the results of the growth kinetics experiment. In addition, the ST cells
showed higher DNA values than LD cells both in DP and WB, which is consistent with the results described above. DNA accumulation increased with increasing FBS concentration in the medium, but this occurred at a higher extent in WB than in DP cells. By 20% FBS DNA synthesis rates were elevated to 180–210% in WB cell cultures and only to 120–140% in DP cell cultures.

In summary, the order (superiority or inferiority) of DP or WB cells in DNA accumulation or DNA synthesis rate was not changed by the FBS concentration within the selected range. However, DNA synthesis rate was stimulated to a higher extent with increasing FBS concentrations in WB cell cultures.

4. Discussion

The comparison of German Landrace pigs as one of the recent domestic pig breeds with wild-type pigs at different stages of postnatal age has revealed substantial differences in growth and quality properties of skeletal muscle between these genotypes. Remarkable differences between wild and domestic pigs in growth and body composition have been reported previously (Bader, 1983; Szentkuti and Sallai, 1988) and are confirmed by this study.

Novel aspects can be derived from this study in terms of the effect of genetic selection on the ontogenetic development of pig skeletal muscle. Surprisingly, DP had formed less muscle fibers during fetal myogenesis than WB both in ST and PM muscle, although their muscle mass was higher at birth. Obviously, selection for postnatal growth and lean accretion did not intensify prenatal myofiber formation, but conversely lead to a lower number of primary and secondary fibers at birth. In addition, skeletal muscle of DP was less mature as seen by lower percentages of type I fibers at birth. Conversion of some secondary fibers adjacent to the primary fiber to type I fibers is indicative of maturity of piglet skeletal muscle (Handel and Stickland, 1987; Rehfeldt et al., 1993; Lefaucheur et al., 1995). Likewise, lower muscular protein, RNA, and DNA concentrations, greater DNA/protein and lower RNA/DNA ratios indicate that the muscle is less differentiated and fiber growth is less advanced in newborn DP than WB.

In principal, the results obtained in the cell culture experiments with porcine satellite cells support the advanced development of WB skeletal muscle. Satellite cell cultures derived from ST and LD muscle of neonatal WB grew faster than that of DP. This was obviously associated with their higher sensitivity to hormones and growth factors contained in serum, which in turn may result from a higher maturity of the endocrine system at birth. Endocrine parameters like growth hormone, IGF-I, sexual hormones, cortisol etc. have been compared between wild and domestic pigs from about 20 to 50 weeks of age (Weiler et al., 1998). Whether and how
the earlier ontogeny of the endocrine system differs between the genotypes remains to be investigated. The response of myogenic cells derived from fast growing vs. slow growing strains of animals has been studied in response of myogenic cells derived from fast growing vs. slow growing strains of animals has been studied in several species such as mice (Rehfeldt et al., 2002), turkeys (Merly et al., 1998), and chickens (Ridpath et al., 1984). In opposite to our results, the cells from the fast-growing strains showed better growth under standard culture conditions, which in parts could be related to higher responsiveness to growth factors like IGF-I (McFarland et al., 1995; Duclos et al., 1996) or EGF (Rehfeldt et al., 2002). Other results obtained with porcine satellite cells suggest that the stage of age, when the cells are taken from the pigs, plays a role for their growth behavior in culture. Thus, Clelland and Stickland (2001) found higher degrees of proliferation in porcine satellite cell cultures from small as compared with large siblings at 4 days of age. However, when satellite cells were derived from 6-week old pigs, opposite results were obtained (Nissen and Oksbjerg, 2006). Hypothetically, at later postnatal stages, when skeletal muscle fibers of domestic pigs grow much faster than that of the wild-type pigs, we would observe a better growth of DP derived satellite cells. This, however, remains to be investigated.

In spite of higher muscle mass in neonatal DP, total protein amount was not different to WB indicating that the proportion of water was much higher in the DP muscles. However, higher total DNA amount in ST (not in PM) muscle of DP at birth may indicate that there is a higher additional capacity for postnatal myofiber formation. Actually, a higher number of additional fibers were formed after birth in DP-ST, but not in DP-PM, where it remained below WB at all stages of age. This is an example of the muscle specificity of the effects of selection for lean growth, which was certainly more effective on an exterior ham muscle (ST) than on the interior tenderloin (PM). Our data on postnatal increase in fiber number are consistent with those from Mascarello et al. (1992) and Lefaucheur et al. (1995), who showed that a third generation of small diameter fibers, which express developmental myosin isoforms, forms shortly after birth. Their results indicated that myofiber formation has not fully ceased at birth. However, it remains to be investigated, whether fiber fragments that already exist at birth, grow in length and thereby become apparent in muscle cross section or whether new fibers are formed by fusion of a third generation of myoblasts. Our results strongly suggest that it is the formation of these tertiary myofibers that has been intensified during selection for lean growth in some muscles.

Nevertheless, postnatal growth in fiber size was accelerated in all DP muscles examined when compared to WB, which is consistent with earlier reports (Bader, 1983; Solomon and West, 1985; Weiler et al., 1995; Fiedler et al., 1998; Müller et al., 2002). In opposite to the first day of age, RNA/DNA was higher and DNA/protein as well as nuclear density were significantly lower (at unchanged protein/RNA) in DP muscles at 7 weeks of age suggesting that protein synthesis has been mainly increased at the level of transcription, which resulted in up to double-sized fibers compared with WB. Subsequently, the differences in fiber size between the genotypes still increased until week 20 ending up with more than double in size in DP than in WB.

In summary, the relative immaturity of DP skeletal muscle in terms of cellular development at birth is followed by an explosive postnatal catch-up growth, which leads to the final superiority of DP muscles in protein accretion. Furthermore, our results provide the evidence that the formation of muscle fibers during prenatal myogenesis has not been stimulated by genetic selection for postnatal (lean) growth. A small contribution to higher ST muscle mass has been realized by additional fiber formation shortly after birth, but in another muscle (PM) fiber number remained lower in DP during postnatal growth. The major difference in muscle mass between DP and WB has been realized through a substantial increase in postnatal protein accretion and myofiber hypertrophy. This is of great importance, because on the one hand, the postnatal potential for lean growth depends on the number of muscle fibers formed prenatally, and on the other hand, extensive myofiber hypertrophy is correlated with adverse effects on stress susceptibility and meat quality (e.g. Fiedler et al., 1999; Karlsson et al., 1999). Thus, so-called giant fibers, which are abnormally large and swollen fibers with signs of degeneration and appear in post mortem muscle as a consequence of extreme fiber hypertrophy, have been observed in domestic, but not in wild-type pigs (Bader, 1983; Solomon and West, 1985; Weiler et al., 1995). The percentage of giant fibers is genetically closely correlated with poor meat quality ($r=0.8$; Fiedler et al., 2004; Rehfeldt et al., 2004). In conclusion, the negative consequences of selection on meat quality may be closely related to the fact, that the progress in lean mass was not realized through a proportional increase in fiber size and number. Moreover, the degree of immaturity of skeletal muscle will be even intensified, when birth weights become lower because of high litter size (Town et al., 2005; Rehfeldt and Kuhn, 2006).

From the results on the distribution of the contractile fiber types a shift to the fast-twitch IIb at the expense of IIA fibers can be concluded for all muscles, which is consistent with results presented by Bader (1983),

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Essen-Gustavsson and Lindholm (1984), and Weiler et al. (1995). The shift to IIb fibers occurs earlier postnatal in LD (7 weeks) than in ST and PM (20 weeks) muscles. Considering the metabolic fiber types, a shift to the proportion of white glycolytic fibers that are poor in mitochondria can be stated for LD and PM muscle, but not for ST muscle. This is due to the fact that more of the IIm fibers in ST showed a medium instead of low oxidative staining in DP than in WB. Essen-Gustavsson and Lindholm (1984) have reported previously, that the contractile type I and IIA fibers can exhibit dark, medium or low NADH staining, and type IIm fibers medium or low NADH-staining. Szentkuti and Schlegel (1985) have also observed differences in fiber type distributions in LD, but not in ST muscle between WB and DP. Probably, the decreased activity level of the wild-type pigs in our study has attenuated the difference to DP in the metabolism of ST muscle that is more involved into movement activity than PM or LD muscles. Differences may also appear later than 20 weeks of age in ST muscle. But altogether, a shift to fast-twitch glycolytic properties of skeletal muscle can be considered as a consequence of the domestication process.

Oxidative metabolism is largely dependent on oxygen supply via an extensive capillary bed; therefore, we investigated whether the shift to glycolytic metabolism in DP was associated with changes in muscular capillary density. Normally, more capillaries are associated with red than with intermediate and white fibers and the fiber area: capillary ratio is smallest in red fibers (e.g., Hather et al., 1991; Degens et al., 1992). This was also observed in the present study. In addition, we found that the capillary density per fiber was almost equal in WB and DP. However, the fiber area supplied by one capillary was larger in DP in all three muscles examined both at 7 and 20 weeks of age. The significance of differences in fiber types and fiber area/capillary are fairly consistent at 20 weeks of age. Conclusively, not the number of capillaries, but increasing diffusion distances represented by increasing fiber size and lower capillary number per fiber area, are probably related to the shift of the myofibers to fast-twitch glycolytic features in DP.

5. Conclusions

Long-time domestication of the pig has lead to substantial changes in the ontogenic development and the contractile and metabolic properties of skeletal muscle. Skeletal muscle of domestic pigs appears less mature at birth and contains a lower number of myofibers compared with wild-type pigs. The later superiority of domestic pigs over wild-type pigs in skeletal muscle mass results mainly from accelerated myofiber hypertrophy and protein accretion at the level of transcription during postnatal growth. To less extent there is a contribution by additional myofiber formation shortly after birth in some muscles. In addition, domestication was associated with a clear shift of skeletal muscle to fast-twitch glycolytic properties.

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