Chapter

Effect of Pig Domestication on Skeletal Muscle Development, Microstructure, and Genetic Mechanism Involved in Myofibre Type Formation

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Abstract

The wild boar and modern highly selected pigs are phenotypically distant European pig breeds reared in contrasting conditions and present ideal model to better understand the mechanisms behind meat quality deterioration related to domestication and selection pressure, which provoked substantial modifications in the ontogenic development as well as contractile and metabolic properties of skeletal muscles. The skeletal muscle of domestic pigs are less mature at birth and contains a lower number of myofibres compared to wild boars; however, expansive myofibre hypertrophy, protein accretion as well as additional myofibre formation are accelerated in the early postnatal period in some muscles in domestic pigs. A comparative view of the cellular and subcellular mechanisms underlying the skeletal myofibre development could help to design a breeding program that would improve the balance between the growth performance, muscularity and meat quality. This chapter therefore outlines the influence of domestication on myofibre formation and differentiation during growth and provides a comparative view on the developmental expression pattern of the MyHC isoforms, the activity of different metabolic enzymes, and the expression of selected genes responsible for the metabolic diversity of the myofibres. Additionally, there is a special emphasis on the type, composition, and histomorphological traits of myofibres.

Keywords: pig, myosin heavy chains, myofibre, immunohistochemistry, qPCR

1. Introduction

The European domestic pig breeds have been raised using the centuries-long process of domestication and artificial selection of its ancestor, the European wild boar (*Sus scrofa scrofa*) (reviewed in [1]). Domestication is considered the biological process of adaptation to management/breeding and results in the selection of unique morphological, behavioural, and production traits. Pig breeds experienced strong selection for specific production traits such as feed conversion

efficiency, carcass composition, lean meat content and muscularity due to changing consumer preferences [1]. Compared with the wild boar, domestic pig breeds have also experienced a substantial increase in the litter size and growth performance. The mean wild boar litter size in Europe is between 4.75 and 6.28 piglets, whereas that in domestic pigs was 10.9 piglets in 1992, which increased to 12.2 piglets in 2001 [2, 3]. In Denmark, it was reported that 14.8 piglets were born alive in 2011 [4]. Similarly, the growth performance comparison between the wild boar and domestic pig showed that the domestic pig grows faster, with their body mass at the age of 5 months being four times larger than that of wild boars. Such immense growth intensity during the postnatal period in the domestic pig has been achieved through exceptional muscle mass accumulation, mostly due to myofibre hypertrophy and protein accretion, which is more pronounced in large white skeletal muscles [5]. Nevertheless, the heavy selection pressure posed on the modern pig breeds has also been associated with some negative/undesirable side effects including those associated with the musculoskeletal phenotype and carcass/meat traits.

The selection for increasing litter sizes leads to a decline in birth weight, thus increasing the incidence of low-birth-weight piglets (body mass < 1 kg at birth). This can be attributed to the intrauterine growth retardation due to insufficient development of the placenta in relation to the number of embryos, which are not sufficiently supplied with oxygen and nutrients [6]. Low-birth-weight piglets can exhibit a lifelong impairment in muscle growth, typically characterised by a decreased number of myogenic cell nuclei and reduced total myofibre number that are larger in diameter [3, 7–9]. Additionally, domestic pig muscles are less mature at birth compared to those of the wild boar [10]; the muscle immaturity could even be intensified in case of a bigger litter size/lower piglet birth weights [11–15]. The effect of a lower birth weight on the carcass and meat traits are inconclusive; however, most studies report that such piglets grow slower, have fatter carcasses, and poorer meat quality (higher drip loss and lower tenderness) (reviewed in [16]).

The selection and changed rearing conditions have also been associated with myofibre hypertrophy and a shift in the muscle myofibre type composition towards the fast-twitch glycolytic state [17, 18] as well as the introduction of certain causative mutations affecting economically important traits. Among the latter is a well-known point mutation in the ryanodine receptor (*RYR1)* gene *(c.1843C > T)* in stress-susceptible pigs [19], frequent in a heavy muscled modern pig breeds such as Pietren and Landrace pigs. It leads to an increased release of calcium from the sarcoplasmic reticulum due to different stress factors to which pigs are exposed. A higher level of calcium in the sarcoplasm induces hyper-contraction and hyper-activation of metabolic processes and can result in a sudden death, which in turn diminishes the meat quality after the slaughter. Due to an altered rate of glycolysis and a rapid fall of pH within the muscle, pale, soft, exudative (PSE) meat is frequently observed after the slaughter in pigs, particularly in the so called white muscles in which fast-twitch glycolytic myofibres dominate in number. Such meat is characterised by an abnormal colour, consistency, and water holding capacity, making the meat dry and unattractive to consumers [20]. This condition can also be observed in the white muscles of highly selected modern pig breeds that are free of the *RYR1* mutation [21]. Extensive myofibre hypertrophy is also associated with the appearance of so-called giant myofibres, which are abnormally large and swollen and appear in *post mortem* muscles as a consequence of extreme hypertrophy, glycogen accumulation, and consecutive intracellular acidity due to excessive glycolysis [11, 15, 22, 23]. Additionally, giant myofibres were frequently observed in the white muscles of domestic pigs, but

rarely in the red muscles or muscles of the wild boar or indigenous pig breeds. The percentage of giant fibres is closely correlated with diminished meat quality traits and lower pH measured 45 min after slaughter (pH_1) [12, 14, 24, 25]. The possible reason for a negative impact of selection on meat quality could be because the increased muscularity was not accompanied with a proportional increase in the capillarisation of the muscle tissue. Accumulating evidences therefore suggest that the domestication and selection of the pig altered the development, growth, and the phenotype of the skeletal muscles to such an extent that meat quality could be compromised.

This chapter outlines the influence of domestication on myofibre formation and differentiation during growth and provides a comparative view on the developmental expression pattern of the MyHC isoforms, the activity of different metabolic enzymes, and the expression of selected genes responsible for the metabolic diversity of the myofibres. Additionally, there is a special emphasis on the type, composition, and histomorphological traits of myofibres.

2. Skeletal myofibre types, their classification, and characteristics

Skeletal muscle tissue is a major contributor to systemic energy homeostasis because of its high rate of energy demand and relatively large mass in comparison to other tissues and represents the biggest part of total body mass in mammals [26]. It is heterogeneous in structure, which results in the different contraction and metabolic properties of muscles. The muscle functional diversity not only allows various motor tasks, such as posture, locomotion, or jumping [27], but is also involved in whole-body energy metabolism as it is the major site for plasma glucose disposal [28]. In mammals, the skeletal muscles are a mixture of functionally specialised myofibre types with different contractile and metabolic properties. The initial classification of muscles/myofibres was based on colour and correlated with contraction speed and fatigability. For example, fast-twitch muscles, which are characterised by glycolytic metabolism and short-lasting but forceful contractions, are generally identified as white muscles. Conversely, slow-twitch muscles, rich in myoglobin and oxidative enzymes are specialised for more continuous or tonic activity and are defined as red muscles [29]; which also more effectively remove glucose from blood than white muscles [28].

Myofibre type diversity primarily depends on the structure of their myosin heavy chains (MyHCs), which are the principal myofibrillar proteins that control myofibre contractile properties. In the locomotor skeletal muscles of adult mammals, one slow (MyHC–I) and three fast MyHC isoforms (MyHC–IIa, –IIx, and –IIb) are generally expressed. MyHCs are responsible for the differences in the myosin ATPase activity and twitch characteristics between myofibre types. The contraction speed of MyHCs increases in the following order: –I < –IIa < –IIx < –IIb [30]. Myofibres may transform through successive steps of MyHC isoform expression i.e. from MyHC–I to –IIa, from –IIa to –IIx, and from –IIx to –IIb, and *vice versa*. In the locomotor skeletal muscles of large mammals such as cats [31], dogs [32], cattle [33], horses [34], and bears [35] only two fast isoforms (–IIa and –IIx) were demonstrated. However, all three fast MyHC isoforms were recognised in pigs [36], llamas [37], and also in some intrinsic laryngeal muscles of different species like the dog [38].

In pig muscles, the fasciculus myofibre type position follows the transition rule –I > –IIa > –IIx > –IIb and proceeded from the centre of the muscle fasciculus to the periphery [39, 40]. During prenatal and postnatal myogenesis, the developmental

MyHC isoforms i.e. embryonic (MyHC-emb) and neonatal (also referred as foetal or perinatal; MyHC-neo) are also expressed. For this reason, hybrid myofibres, which contain more than one MyHC isoform are present in muscles and are numerous above all during myogenesis and early postnatal development or under the condition when different factors trigger the functional adaptation of the muscle such as exercise, changed endocrine status, neuromuscular stimulation, and inactivity.

The metabolic profile of the myofibres generally corresponds to the energetic demands of each MyHC. A greater oxidative capacity is characteristic for tonic or

		Myofibre type			
		I	IIa	IIx	IIb
Antibody	NLC-MHCs	++	—	—	—
	SC-71	—	++	+	—
	BF-35	+	+	—	—
	BF-F3	—	—	—	++
SDH		++	++/+	+	—

+ and ++ denote moderate and strong positive reactions, respectively. +/– denotes weak reaction and – denotes a negative reaction.

Table 1.
Immunohistochemical and succinate dehydrogenase (SDH) activity-based classification of myofibre types. Immunoreactivity of antibodies frequently used for myofiber type classification in pig muscles are presented.

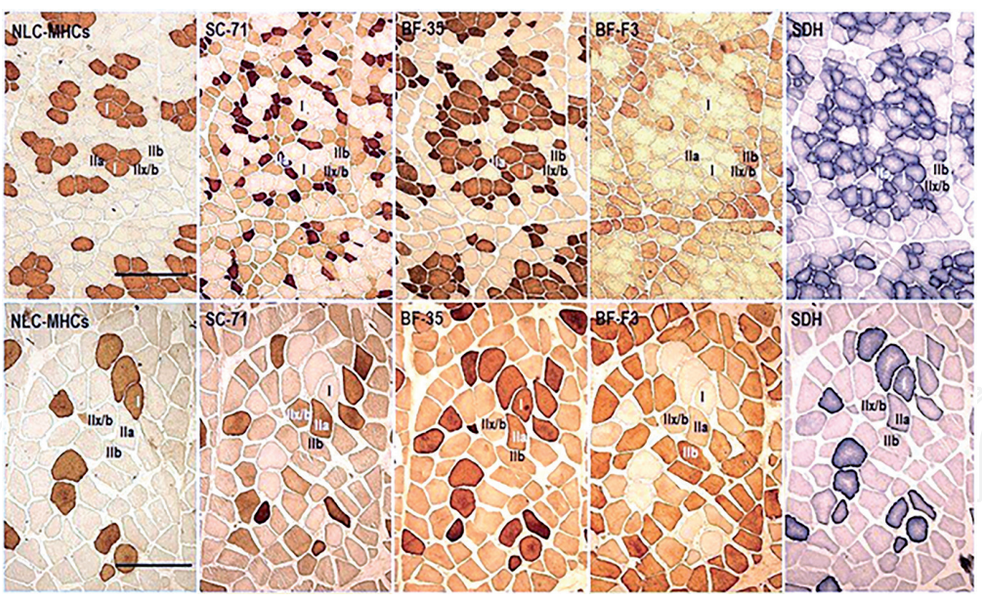

Figure 1.
Antibody reactivity and SDH activity in the wild boar and domestic pig. Serial transverse sections of the longissimus dorsi (LD) muscle from the wild boar (upper row) and domestic pig (lower row) stained with the monoclonal antibodies NLC-MHCs, SC-71, BF-35, and BF-F3. Slow-twitch myofibres (type I) react with the NLC-MHCs antibody and all remaining unstained myofibres are fast-twitch type II myofibres. In the pig, the SC-71 antibody recognises both MyHC–IIa and MyHC–IIx myofibres; however, it has a higher affinity for MyHC–IIa. BF-35 recognises all MyHC isoforms, except MyHC–IIx and –IIb in the pig skeletal muscle. The BF-F3 is specific to MyHC–IIb myofibres. BF-35 negative myofibres are divided into two subgroups: Pure IIb myofibres (strongly stained with BF-F3) and hybrid IIx/b myofibres (weakly stained with BF-F3). Succinate dehydrogenase (SDH) activity demonstrates the oxidative potential of myofibres. Type I and IIa myofibres are intensively stained (highly oxidative) in both the wild and domestic pig, IIx and IIx/b myofibres are stained moderately and weakly, respectively and IIb myofibres are negative. Also, the difference in the myofibre diameter between the wild boar and domestic pig can be noted (cf. panels in upper and lower row). Scale bar, 250 μm is valid for all panels. (from Fazarinc et al., 2013 [17]. Reprinted by permission of Slovenian veterinary research).

slow-contractile type I myofibres, which contain a higher amount of lipids as the main energy source. The IIb myofibres are predominantly glycolytic and use glycogen for strong, transitory contractions, whereas IIa and IIx myofibres represent the metabolically intermediary type between I and IIb myofibres [41]. Thus, the myofibre specific contractile and metabolic phenotype that correlates with MyHC expression and metabolic enzymes activity enabled an advanced immuno–/enzyme histochemistry-based classification of myofibres that relies on the specificity of the immunoreactivity of antibodies for different MyHC isoforms and histological assessment of metabolic enzyme activities as exemplified in **Table 1** and shown in **Figure 1**.

3. Proportion of different types of myofibres and their histomorphometric traits

The MyHC expression pattern, which largely determines the proportion and myofibre contractile and metabolic profile (e.g. glycogen and lipid contents), also correlates with myofibre histomorphometric traits and consequently, the muscle to meat conversion and meat quality traits [42, 43]. In pigs, the oxidative and metabolic intermediate myofibres that express MyHC–I, –IIa, and –IIx are associated with desirable meat quality traits, such as the water-holding capacity, pH, and tenderness (reviewed in [44]). However, the domestication and breeding selection are associated with myofibre hypertrophy and changes in myofibre type composition with an age-dependent increase in the proportion of fast glycolytic myofibres [13, 15, 45]. In the white muscles of the domestic pig, the glycolytic MyHC–IIb positive myofibres prevail and are located in the peripheral region of the muscle fasciculus [36]. It was initially presumed that in the domestic pig, the MyHC–IIb isoform expression is related to genetic improvement and breeding; however, the presence of IIb myofibres was also demonstrated in the wild boar [17], albeit their number was sustainably lower as demonstrated by the difference of BF-F3 immunopositively stained myofibres in **Figure 1**.

The ratio of preferentially oxidative myofibre types is higher in the wild boar as well as some indigenous pig breeds than in the selected modern domestic pig breeds [10, 46–48]. The comparison of growing wild boars and domestic pig also revealed substantial differences in the direction and intensity of postnatal MyHC transformation and myofibres' hypertrophic potential in the longissimus dorsi muscle [10, 49]. The MyHC differentiation is accompanied by the thickening of all myofibre types. Myofibre hypertrophy is especially intense in the period from 3 weeks to 7 months of age. In the domestic pig, the hypertrophy of all myofibre types is more intense than in the wild boar, especially that of MyHC–IIx and –IIb myofibres. The intensity of myofibre thickening markedly declines after 7 months of age [10]. The differences in the myofibre type, composition, and hypertrophic potential schematically presented in **Figure 2** are based on the observations from our comparative studies [10, 17]. When the sizes of different myofibre types of domestic pigs and wild boars are compared, the cross-sectional area (CSA) of IIb myofibres is markedly larger than CSA of type I and IIa myofibres in the domestic pigs. The difference in the CSA areas of IIb and IIx myofibres is not so distinctive. In the wild boar, the sizes of all myofibre types are closer to each other. In the early postnatal period, the differences in CSA between the myofibre types are smaller, but when the pigs reach slaughter weight, the CSA of type IIb myofibres fibres is largest, especially in the white muscles. Notably, type IIb myofibres thickened faster than other myofibre types as a consequence of selection based on enhanced lean content which favours larger type IIb myofibres. The MyHC–IIb is the

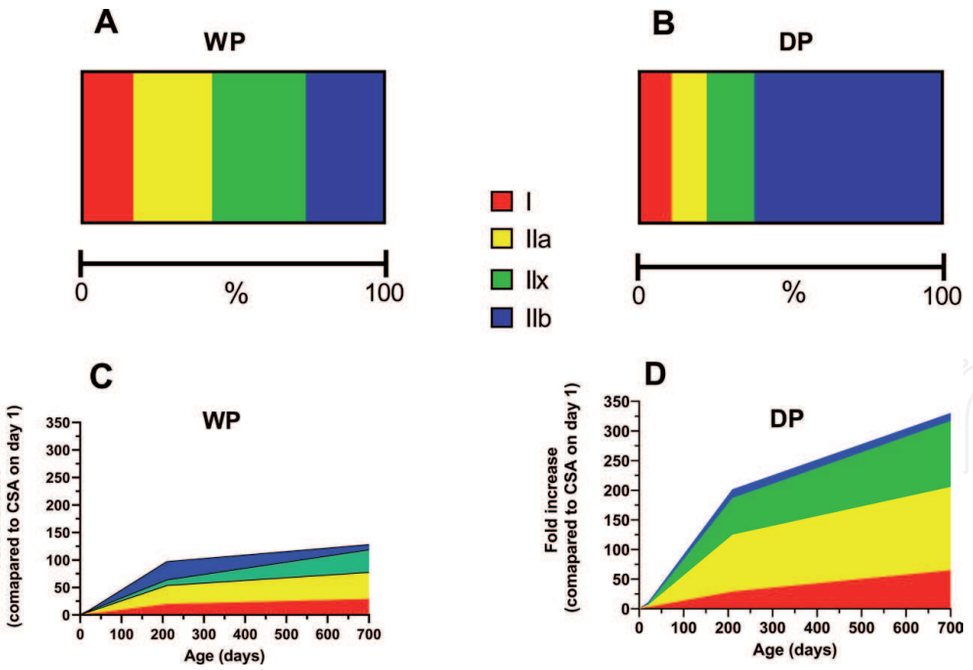

Figure 2.
Composition and hypertrophic potential of myofibre types in the longissimus muscle of the wild boar (WB) and the domestic pig (DP). (A, B) differences in the muscle myofibre type composition between WB (A) and DP (B) at the age of 2 years. Note that the proportion of oxidative type I, IIa, and IIx myofibres is significantly higher in the longissimus muscle of the WP (~65%) than in the DP (~38%). Contrarily, the proportion of glycolytic IIb myofibres was more than 2-fold higher in the DP (> 60%) than in the WB (~25%). (C, D) differences in the myofibre hypertrophic potential in WB (C) and DP (D) from birth to adulthood. Given are the values of fold increase in the cross-sectional area (CSA) compared to the CSA of myofibres I on day 1 in the case of type I myofibres. Hypertrophic potential of type IIa, IIx, and IIb myofibres was calculated relative to the CSA of type IIa myofibres on day 1.

predominant isoform in the white muscle such as longissimus dorsi muscle, especially in the domestic pig and is the main factor that contributed to the increase in muscle mass. The co–expression of the MyHC–IIb and the MyHC–IIx is frequently regarded as the fine regulation of IIx and IIb gene expression in white muscles and is primarily influenced by the breed [50]. The assumption that the genetic background is a crucial determinant of muscle characteristics was further confirmed by a recent comparative study of young pigs of the Iberian and conventional breeds reared under identical conditions, the Iberian pigs had a higher intramuscular fat (IMF) content and oxidative metabolism in the longissimus dorsi muscle [51].

Contrastingly, the published data about the proportion of type I myofibres in the domestic pig and the wild boar are contradictory. In some studies, a considerably higher proportion of type I myofibres was observed in the wild boar than in the domestic pig [10, 52]. On the contrary, other studies report that type I myofibres are even more numerous in the domestic pig than in the wild boar [5, 13]. Notably, the detection of type I myofibres is reliable, regardless of whether immunohistochemistry or myosin ATPase-based methods are used. Therefore, the reported differences cannot be attributed to the techniques used to identify type I myofibres and are probably triggered by rearing conditions. Recent studies also revealed that the muscle MyHC composition is influenced by additional factors, such as animal nutrition, physical activity and environmental temperature [39, 46]. Whereas domestic pigs are kept in farm breeding conditions and fed with typical commercial diet *ad libitum*, the wild boar is highly physically active on a daily basis as they ransack for food. This is probably the main reason for a higher percentage of slow twitch type I myofibres in the wild boar than that found in some other studies in which wild boars were kept in group housing with diminished locomotor activity and were fed *ad libitum* or when samples were obtained from animals kept in a

zoological garden [13, 53]. Endurance exercise increases the proportion of oxidative myofibres along with the mitochondrial respiration of fatty acids, and our recent study with the Krškopolje pig, a Slovenian autochthonous breed, also showed the effect of the production system on the myofibre type composition; pigs reared in organic systems with outdoor space (spontaneous physical activity) have a myofibre composition shifted towards the oxidative (SDH-positive) phenotype [46].

Myofibre metabolism largely depends on the oxygen supply via an extensive capillary bed. Normally, a large number of capillaries are associated with oxidative myofibres and the ratio between the myofibre CSA to capillary is smallest in type I myofibres [54]. The capillary density per myofibre is almost equal in the wild boar and domestic pig. However, the myofibre area supplied by one capillary is larger in the domestic pig. IIb myofibres also tend to have lower numbers of mitochondria per unit area and are thus less oxidative and utilise glycogen to a greater extent than the red muscles, resulting in lactate accumulation in the muscles. When the myofibre CSA increases, the number of capillaries per area decreases, consequently, the extraction of lactate from myofibres is hindered. Therefore, pigs with smaller IIb myofibres in the white muscles should be selected, to overcome lactate diffusion problems in white muscles [45]. Conclusively, it is not the number of capillaries, but increasing diffusion distances due to increased myofibre size and lower capillary number per myofibre area, are related to the shift of the myofibres to fast-twitch glycolytic muscle characteristics in domestic pigs [5].

4. Developmental pattern of myofibre type formation

The typical arrangement of myofibre types in the fasciculus muscle of adult pigs is defined predominantly by prenatal development of the primary and secondary myofibres. The primary myofibre acts as a centre around which the myoblasts align and fuse to form the secondary myofibres. This process results in a unique distribution of myofibres consisting of clusters of type I myofibres surrounded by the fast type II myofibres. During the prenatal period, the primary myofibres express slow MyHC–I, whereas the fast secondary myofibres primarily express developmental MyHC isoforms. In the early postnatal period, the expression of MyHC–I also starts in the secondary myofibres that are in contact with the primary myofibres, meaning that they are transforming into type I myofibres, whereas the remaining secondary myofibres mature into MyHC–IIa, –IIx, and –IIb [40]. In the early postnatal period, the expression of slow MyHC–I increases and that of developmental MyHCs diminishes in the secondary myofibres that surround the centrally positioned primary myofibres [40]. The expression of the developmental isoforms of MyHC decreases towards the end of gestation, when they are substituted by the adult fast MyHCs in the following sequence: embryonic/neonatal > IIa > IIx > IIb. MyHC–IIa and –IIx are already co-expressed in the secondary myofibres at birth, whereas the MyHC-IIb first appears during the early postnatal period [55]. Regarding metabolic phenotypes, all myofibres are oxidative at birth. However, in parallel with the shift to the expression of adult MyHCs that occurs during the first postnatal weeks, a metabolic switch occurs as they differentiate into oxidative, oxidative-glycolytic, or glycolytic myofibres [56]. In the one–day–old wild boar, the proportion of transitional I/IIa myofibres is significantly higher than in the domestic pig (**Figure 3**), although there are no differences in the proportion of pure type I myofibres between both breeds [10]. This observation could indicate that the transformation of secondary (type II) into slow type I myofibres is faster in the longissimus dorsi muscle of the wild boar than the domestic pig at birth. During the early postnatal period, all three adult fast MyHCs (–IIa, –IIx, –IIb) are sequentially expressed. In this age period, the

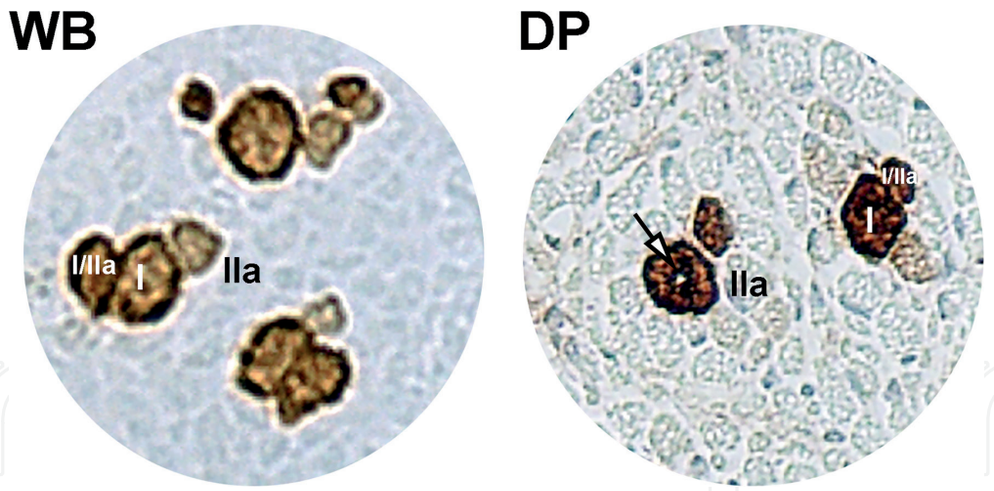

Figure 3.
Longissimus dorsi muscle of a one-day-old wild boar (WB) and a domestic pig (DP) stained with the monoclonal antibodies NLC-MHCs specific for slow-twitch MyHC–I. Positive immunostaining is observed in type I myofibres that originate from the primary myofibres and are located in the centre of the muscle fasciculus. Type I myofibres are surrounded by a subpopulation of smaller secondary myofibres (I/IIa myofibres). Type IIa myofibres are immuno-negative. Individual type I myofibres of domestic piglets still have a hollow centre (arrow) indicating the end of the myotube phase in myofibre formation.

co–expression of two MyHCs in the same myofibre is frequent, indicating that the myofibre functional specialisation is intense. In the longissimus dorsi muscle of the wild boar, the distinct shift towards myofibres expressing MyHCs –I, –IIa, and –IIx is detected, whereas the number of MyHC–IIb-positive myofibres prevail in the domestic pig [10].

After birth, thermogenesis in piglets almost exclusively depends on muscle shivering. Piglets raised in the natural habitat experience exhibit an increased exposure to the cold than those raised in farm conditions with lamp heating. Cold exposure during the early postnatal period and the consequent muscle activity (shivering) dramatically increases the expression of MyHC–I and decreases the neonatal MyHC [57]. This is the most likely explanation for the accelerated myofibre transformation towards type I observed in the wild boar during the first hours *post–partum*. Muscle thermogenic adaptation in terms of MyHC composition was also described in older pigs. An increased percentage of type I myofibres in pig muscles has been observed after prolonged (few months) cold exposure [57, 58]. Therefore, thermoregulation in the early postnatal period in combination with a higher physical activity is likely the main environmental factor triggering the myofibre transformation towards oxidative myofibres in which MyHCs –I, –IIa, and –IIx were predominantly expressed in the wild boar. Faster myofibre maturation in the wild boar was also confirmed with the antibody against foetal MyHC [10]. In the wild boar, the myofibres in the periphery of the muscle fasciculus were less intensely stained than in the domestic pig; thus, this provided additional evidence that the process of MyHC transformation from developmental to adult MyHCs is accelerated in the wild boar. Strong immunohistochemical reactions were mostly restricted to the myofibres that were in the vicinity of type I myofibres, suggesting that foetal MyHC expression progresses from the centre towards the periphery of the muscle fasciculus in the early postnatal period. A decrease in the foetal MyHC is first observed in myofibres in which expression of MyHC–IIx and –IIb started, followed by IIa myofibres. Hence, the transformation of type II myofibres into type I, the decline in the expression of developmental MyHC, and its subsequent replacement with the adult fast MyHCs isoforms are strong criteria for the estimation of myofibre differentiation in the process of postnatal muscle development.

The determination of foetal MyHC isoforms can also present a useful model to investigate the hyperplastic muscle potential during postnatal growth. Small irregularly scattered foetal MyHC–positive myofibres are present in the first postnatal weeks in pig muscles and are designated as third-generation myofibres. The third-generation myofibres had a very small diameter and are randomly scattered among the normal-sized myofibres and displayed a positive immunohistochemical reaction with antibodies against foetal MyHCs (**Figure 4**). These data suggested that the total myofibre number in pig is determined after birth, mainly in the first postnatal weeks [40, 59]. This myofibre population are more numerous in the domestic pig than the wild boar [10] and could contribute to an extraordinary muscle growth potential in domestic pig breeds and probably contributes to the *post–natal* increase in the total myofibre number. Isolated small foetal MyHC–positive myofibres are observed in pigs in the later growth period and probably represent activated satellite cells [10, 60]. Nevertheless, their number in older pigs (7 months) is probably too low to substantially contribute to the *post–natal* myofibre hyperplasia. Therefore, we assume that the estimation of the post-natal effect of foetal MyHC–positive myofibres on the hyperplastic muscle growth potential in the wild boar or the domestic pig is limited after the first postnatal weeks. It is obvious that the disappearance of foetal MyHC is much faster in the wild boar than in the domestic pig (**Figure 4**). Evidently, domestication and selection of the pig has substantially changed the ontogenic development of the pig skeletal muscles, especially the white muscles.

The differences in the MyHC transformation pattern/muscle development between the wild boar and domestic pig were additionally confirmed with the qPCR analysis of *MyHC* isoforms at the mRNA level [49]. The most significant changes in the *MyHC* transcript levels in the longissimus muscle in both breeds occurred during the initial three postnatal weeks. From 1 day to 3 weeks postnatal, the order of *MyHC* transcript levels changed in the following order: *IIx* > *IIa* > *I* > *embry* > *IIb* to *IIx* > *IIb* > *IIa* > I > *embry*. Although this pattern of postnatal transition was identical in both breeds, the quantitative changes were substantially different between the breeds. $MyHC_{embry}$ expression disappeared approximately 10-fold faster in wild boars than domestic pigs, whereas *MyHC–IIb* increased to substantially

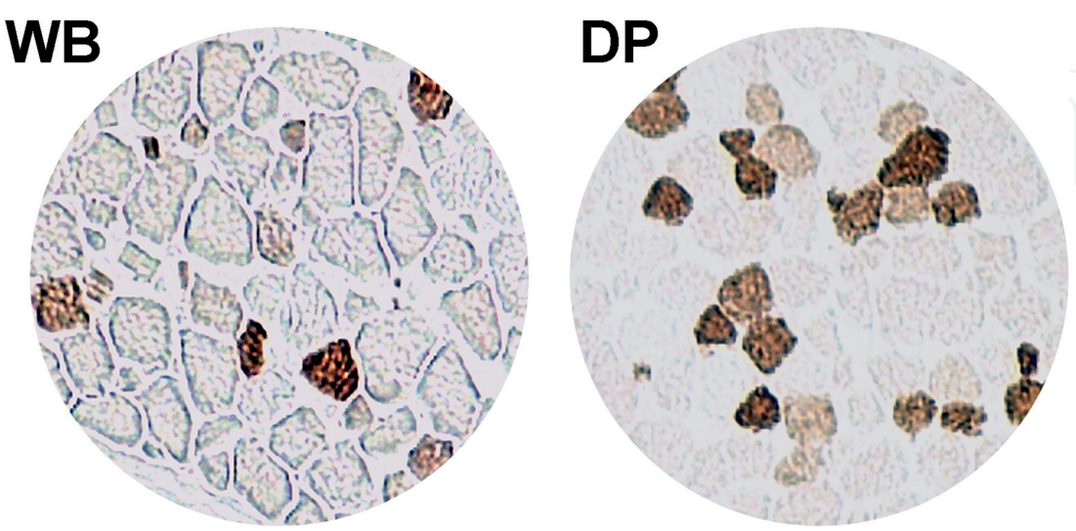

Figure 4.
The longissimus dorsi muscle of a 3-week old wild boar (WB) and a domestic pig (DP) stained with the monoclonal antibody F158.4C10 against the foetal MyHC. The number of immunopositively stained myofibres (pale to dark brown) still expressing foetal MyHC is higher in the DP than in the WB. Additionally, immunopositive myofibres with a very small diameter, representing the third generation of myofibres, are present.

higher levels in domestic pigs. A positive correlation between the relative amount of each individual *MyHC* mRNA and its corresponding protein in the longissimus dorsi muscle has been demonstrated in growing commercial crossbred pigs [61]. These results support the hypothesis that the *MyHC* genes are directly transcriptionally regulated in porcine skeletal muscles [56, 62]. One of the developmental MyHCs that prevail during gestation *is* $MyHC_{embry}$. Developmental MyHCs are then replaced by adult fast MyHCs (i.e. MyHC-IIa, –IIx, and –IIb) in the late gestation/early postnatal period. The $MyHC_{embry}$ expression level thus reflects muscle maturity. $MyHC_{embry}$ was also reported in 6-week-old domestic pigs [63] and could be associated with slower muscle maturation or appearance of the third generation of myofibres, which could contribute to myofibre hyperplasia in the muscles of domestic pig [63]. The higher *MyHC–IIx* expression level observed in newborn wild piglets provided additional evidence that myofibre maturation and the replacement of developmental with adult MyHC was accelerated during the first post-partum hours in 1-day-old wild piglets.

5. Hyperplastic potential of pig muscles

In pigs, the skeletal muscle growth potential could be also characterised by the dynamics of age-related changes in satellite cell proliferation and differentiation. Postnatally, satellite cells play a crucial role in the muscle development, growth, and regeneration [64, 65]. They provide the cell nuclei for muscle fibre fusion and growth and are able to terminally differentiate into myofibres. Adult satellite cells are mostly quiescent and express the transcription factor Pax7 and Myf5 when they enter into the myogenic phase [66–68]. Activated satellite cells undergo proliferation and subsequently, myogenic differentiation to finally form new myofibres or fuse with existing myofibres [65]. In addition to Pax7 and Myf5, the proliferating myoblasts start to express MyoD (myoblast determination protein 1). The myocytes downregulate Pax7 and upregulate the differentiation marker myogenin (MyoG) (reviewed in [64]). During the first postnatal weeks, up to 60% of cells isolated from piglet muscle belong to the satellite cell population and 90% of them are in a state of proliferation [65]. Between the weeks 7 and 21, the percentage of satellite cells lowers dramatically and in adult pigs only 2–5% of cell nuclei belong to the satellite cells [69]. The size of the satellite cell pool established during early development is crucial for the lifelong muscle performance [68]. Additionally, these data indicate that a high percentage of satellite cells remain proliferative during the early rapid postnatal muscle growth and probably represent a third-generation of myofibres. Satellite cell differentiation is likely to modulate the muscle growth because it regulates the accretion of DNA in muscle fibres as well as the number of satellite cells that remain capable of proliferation. Quinn et al. [70] demonstrated that embryonic myoblasts isolated from foetal calves with the double-muscled phenotype display a delay in differentiation compared with myoblasts isolated from normal foetuses. A similar discrepancy in differentiation and formation of the myofibres between the wild boar and domestic pig was confirmed with *in-vitro* growth kinetics of muscle satellite cell cultures derived from domestic and wild-type pigs and analysed by changes in DNA and protein content. Domestic pig muscles exhibited lower numbers of myofibres and were less mature at birth, as seen by DNA, RNA, and protein composition, whereas the satellite cell from the neonatal muscles of wild boar showed intense growth. Contrary to the observation on the first day of age, the RNA/DNA ratio was higher and DNA/protein as well as nuclear density were significantly lower (at unchanged protein/RNA) in domestic pig muscles at 7 weeks of age, suggesting that protein synthesis has mainly

increased at the transcriptional level, resulting in excessive myofibre hypertrophy in the domestic pig [5].

We can conclude that relative immaturity of domestic pig skeletal muscle in terms of cellular development at birth is followed by an explosive postnatal myogenesis, leading to the final superiority of domestic pig muscles in protein accretion. Furthermore, a small contribution to higher muscle mass has been realised by additional myofibre formation shortly after birth but mostly in large skeletal muscles such as the semitendinosus muscle. Therefore, the major difference in muscle mass between the domestic pig and wild boar has been realised through a substantial increase in the postnatal protein accretion and myofibre hypertrophy [5].

6. Expression patterns of genes involved in myofibre metabolism

The expression patterns of genes that regulate different aspects of energy utilisation are becoming of significant value in pig muscle studies. On the basis of an increased expression of MyHCs –I, –IIa, and –IIx and parallelly increased SDH activity in the myofibres of wild boars, certain differences in the expression of the genes involved in the energy metabolism in the myofibre are expected. The glycolytic cycle is particularly important for lactate formation in the muscle. The phosphorylation of glucose to glucose-6-phosphate is controlled by hexokinase (HK), which is the key enzyme in the glycolysis reaction, and HK2 is a predominant enzyme form found in the skeletal muscles [71]. Another promising candidate gene for traits related to skeletal muscle metabolism in pigs is the glycogen synthase gene (*GYS1*), which encodes glycogen synthesis in skeletal muscles [72]. The mRNA levels of these two genes (*HK2* and *GYS1*) were significantly higher in fast-glycolytic-type muscles than slow-oxidative-type pig muscles [73], and the expression level of *GYS1* gene in pigs were significantly lower in pigs with a higher post-mortem pH_1 and pH measured 24 hours after slaughter (pH_{24}). In our study, we did not demonstrated any differences between wild boars and domestic pigs in the expression of these two enzymes at the mRNA level or any correlation between both pHs (pH_1 and pH_{24}) and the glycolytic enzymes genes expression [49]. We found minor positive correlations in the expression of genes for IIb MyHC and Gys1 ($p = 0.0735$), which is in accordance with a greater glycolytic potential of IIb myofibres.

Recent studies have also focused on the genes that are important for different lipid metabolic processes in myofibres. One of these genes is a *peroxisome proliferator-activated receptor gamma coactivator 1 alfa* (*PGC-1α*) that regulates downstream target genes and has a crucial role in myofibre metabolism and type maturation and can even induce the transformation of fast myofibres into the slow type [74]. *In vitro* evidence suggests that *PGC-1α* also plays a role in myoblast differentiation [75]. Additionally, other lipid metabolism-related genes have attracted research interest because of their potential association with meat quality, especially those involved in lipogenesis, fatty acid uptake and fatty acid oxidation [47, 48, 76].

The *MyHC* expression patterns observed in the wild boar and domestic pig were in agreement with the higher number of SDH-positive myofibres, and higher intra-myofibre lipid (IMFL) content (estimated by oil red O staining intensity) [49]. These data corroborate with previous studies reporting a positive correlation between the abundance of *MyHC-I* transcript and SDH activity [61] or the IMF content [47] and a negative correlation between *MyHC-IIb* and the IMF levels [77]. It can be assumed that the relative abundances of MyHCs are interdepended with the abundances of lipid metabolism-related genes. PGC-1α has a clear impact on

metabolism via its effects on several downstream target genes that have an impact on lipid metabolism in pigs, as established by Erkens et al. [76]. Similarly, it was demonstrated that *PGC-1α* expression is higher in local Chinese pigs, together with higher *MyHC–I*, *–IIa*, and *–IIx* transcript levels, higher IMF levels, and superior meat quality than those of Landrace pigs [47, 48]. The *PGC-1α* level is higher during the early postnatal period in the wild boar than in the domestic white pig; however, the difference in expression between the breeds was not significant in adult pigs. The relative *PGC-1α* expression level diminished during the early postnatal weeks in both breeds, during which the myofibres underwent intensive contractile and metabolic specialisation. The level was maintained in the adult wild boar, whereas it increased after the first three weeks in the domestic pig. The latter observation can be explained by the finding that PGC-1α plays a role in the myoblast differentiation/maturation [75]. In addition, the mRNA expression levels of lipogenesis (e.g. *peroxisome proliferator-activated receptor gamma, PPARγ*)- and fatty acid uptake (e.g. *lipoprotein lipase, LPL*)-related genes were shown to be higher in local fatty pig breeds i.e. Rongchang breed in China compared to the commercial Landrace pigs, whereas the expression of the fatty acid oxidation-related gene (*carnitine palmitoyltransferase-1B, CTP-1B*) was higher in Landrace pigs [48]. We did not find any considerable differences in the mRNA expression of examined enzymes between the wild boar and domestic pigs of the same age; however, we did note a few age-related changes. This was rather unexpected, but it corroborated the results of Park et al. [62], who also provided evidence that energy metabolism and the contraction speed could be uncoupled in myofibres. To summarise, the metabolic features of pig skeletal muscle, SDH activity, and the oil red O IMFL staining intensity demonstrated a clear relationship with the contractile profile reflected by MyHC expression, whereas unexpectedly there is no correlation with the expression of genes involved in lipid uptake and utilisation.

7. Conclusion

To summarise, the comparison of growing domestic pig with wild ancestors of the same age show that domestication and breeding conditions lead to changes in the direction and intensity of postnatal MyHC transformation in pig longissimus muscles. In the domestic pig, the transformation of MyHC was shifted towards myofibres that expressed MyHC–IIb, which resulted in accelerated myofibre hypertrophy and protein accumulation with clear glycolytic metabolic properties of the muscle as a whole. In the wild boar, the maturation of the longissimus muscle is characterised by a faster elimination of developmental MyHC and differentiation towards oxidative and metabolic intermediate myofibres in which type I, IIa, and IIx MyHCs prevailed. The histochemical analysis of oxidative enzyme activity and intramyofibrilar fat content corroborates with the MyHC expression profile. However, the mRNA expression level of the studied genes involved in glycolysis, lipid uptake, and lipid utilisation did not differ between the wild and modern domestic pig breeds, suggesting that posttranscriptional modifications regulate the metabolic activity of these enzymes.

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