The impact of housing conditions on porcine adult stem cell populations differ between adipose tissue and skeletal muscle

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Abstract

Background. In pigs, the ratio between lean mass and fat mass determines production efficiency and is strongly influenced by the number and size of cells in tissues. During growth, the increase in the number of cells results from the recruitment of different populations of multipotent adult stem cells residing in the tissues. We hypothesized that the impact of a hygiene challenge on the proportions of adult stem cells in adipose tissue and skeletal muscle may differ between pigs with different residual feed intake (RFI), a measure of feed efficiency.

Methods. At the age of 11 weeks, Large White pigs from two lines divergently selected for low and high RFI were housed in two contrasting hygiene conditions (good *vs* poor). After six weeks of challenge, pigs were slaughtered (n = 5-9/group). Samples of subcutaneous adipose tissue and *longissimus* muscle were collected, and cells from the stromal vascular fraction (FSV), which includes adult stem cell populations, were isolated from each tissue. Adipose and muscle cell populations from the FSV were phenotyped by flow cytometry using antibodies that targeted different cell surface markers (CD45 to identify hematopoietic cells; CD34, CD38, CD56 and CD140a to identify mesenchymal stem cells (MSC) with adipogenic and/or myogenic potential).

Results. Adipose tissue and muscle shared some common MSC populations although MSC diversity was higher in muscle than in adipose tissue. In muscle, the $CD45^{-}CD56^{+}CD34^{-}CD140a^{+}$ and $CD45^{-}CD56^{+}CD34^{+}CD140a^{+}$ cell populations were abundant. Of these two cell populations, only the proportions of $CD45^{-}CD56^{+}CD34^{+}CD140a^{+}$ cells increased ($P \leq 0.05$) in pigs housed in poor hygiene compared with good hygiene conditions. For the $CD45^{-}CD56^{-}CD34^{-}$ cell population, present in low proportion, there was an interaction between hygiene condition and genetic line ($P \leq 0.05$) with a decrease in low RFI pigs housed in poor hygiene conditions. In adipose tissue, the two abundant MSC populations were $CD45^{-}CD56^{-}CD34^{-}$ and $CD45^{-}CD56^{+}CD34^{-}$. The proportion of $CD45^{-}CD56^{-}CD34^{-}$ cells increased ($P \leq 0.05$) whereas the proportion of $CD45^{-}CD56^{+}CD34^{-}$ tended to decrease ($P \leq 0.1$) in pigs housed in poor conditions. This study shows that the proportions of some MSC populations were affected by hygiene of housing conditions in a tissue-dependent manner in pigs of both RFI lines. Therefore, these cell populations could be targeted to modulate growth and body composition in growing animals.

Introduction

During postnatal growth and development, farm animals like pigs are exposed to a diversity of environmental stimuli (Colditz et al., 2016). Stressful situations like transport and housing in poor hygiene conditions can result in immune system hyper-activation. The animal ability to cope with these different challenges will influence the growth of their productive tissues and subsequently their growth efficiency and robustness. Fat animals are generally less efficient in the conversion of feed into meat (Sillence, 2004). Moreover, as excess body fat triggers the production of immune and inflammatory cells, the lean-to-fat ratio in the body may influence the ability to fight against infection, and thus the robustness of animals (Patience, Rossoni-Serão & Gutiérrez, 2015). Hence, a better control of body composition in growing pigs requires a better understanding of the dynamics and flexibility of muscle and adipose tissue growth when the environment surrounding pigs is modified. The mass of skeletal muscle and adipose tissue is largely determined by the number and size of muscle fibers and adipocytes (Lefaucheur, 2010; Louveau et al., 2016). This increase in cell number results from recruitment of multipotent mesenchymal stromal/stem cells (MSCs) resident in tissues (Dominici et al., 2006). These cells comprise heterogeneous populations of undifferentiated cells able of self-renewal with the capacity to differentiate into cells of the mesenchymal lineage (adipocytes, myocytes or osteocytes for instance). These MSCs have now emerged as important targets to control growth and final body composition in livestock (Johnson, 2013; Dodson et al., 2015).

The identification of MSC populations is based on the use of a combination of cell surface markers and flow-cytometry (Bourin et al., 2013; Relaix et al., 2021). Thus, MSCs are positive for CD105, CD90, CD73 and negative for hematopoietic markers CD11b, CD14, CD34 and CD45. More specifically, both skeletal muscle and adipose tissue cells include resident MSCs that share common cell surface markers. For example, the CD56 marker, also known as a neural cell adhesion molecule (NCAM) and a myogenic marker in muscle (Pisani et al., 2010a; Wilschut et al., 2011; Uezumi et al., 2014), has been also found in MSCs from porcine adipose tissue. Recently, the phenotypic marker CD140a, or platelet derived growth factor receptor alpha (PDGFRα) emerged as an important MSC marker. It has been shown that CD140a^{+} cells derived from muscle or adipose tissue have adipogenic potential (Lee & Granneman, 2012, Uezumi et al., 2010, 2016; Sun, Berry & Olson, 2017). Finally, CD38, one of the main NAD-degrading

enzymes in mammalian tissues, has been suggested as a novel marker of committed preadipocytes in mice (Carrière et al., 2017). Other studies have shown that the combination of CD34 and CD56 markers allow the discrimination between myogenic and adipogenic progenitors in human and pig muscle (Pisani et al., 2010a,b; Perruchot et al., 2013). Taken together, these cell surface markers could be used to decipher the functional signatures of muscle and adipose tissue.

Thus, despite the well-known relationship between adipose tissue and skeletal muscle throughout life (Crisan et al., 2008; De Carvalho et al., 2019), there is a notable lack of studies investigating deeply these two tissues. In this context, we propose to characterize and compare MSCs populations from adipose and skeletal muscle tissues in response to an environmental challenge in two pig lines divergently selected for feed efficiency (Gilbert et al., 2007). With a genetic selection mainly based on production criteria for more than 50 years, pigs are potentially more susceptible to infectious diseases and have a weaker immune response (Knap & Doeschl-Wilson, 2020). In order to determine if genetic selection has an impact on the immune response of the best performing pigs, a sanitary challenge (good *versus* poor hygiene of housing conditions) was recently carried out on two lines of pigs divergent for their residual feed intake (RFI), a measure of feed efficiency (Chatelet et al., 2018). It has been shown that this sanitary challenge reduced the growth performance of pigs and stimulated their immune system (Le Floc'h et al., 2014). We hypothesized that hygiene of housing conditions will impact the proportions of hematopoietic and/or MSC cells in subcutaneous adipose tissue (SCAT) and skeletal muscle and that the response may differ between pigs with different RFI.

Materials & Methods

Ethical approval/declaration

The experiment was performed in the INRAE UE3P experimental facility (Saint-Gilles, France; (doi.org/10.15454/1.5573932732039927E12) in compliance with the ethical standards of the European Community (Directive 2010/63/EU) and was approved by the regional ethical committee (Comité Rennais d'Ethique en matière d'expérimentation animale or CREEA Rennes). The experiment approval number is APAFIS#494–2015082717314985.

Animals

For this experiment, a subset of pigs was selected from the period 1 of a larger study previously described by Chatelet et al. (2018). Growing Large-White pigs (males and females) originated from the 8th generation of two lines divergently selected for residual feed intake (RFI), a measure of feed efficiency (Gilbert et al., 2007). Briefly, animals from the high RFI (HRFI) and the low RFI (LRFI) lines were weaned at 4 weeks of age. At 11 weeks (≈ 25 kg), half of the LRFI and HRFI pigs were then assigned to either a room with good hygiene conditions and the other half to a room with poor hygiene conditions. Good housing conditions included room cleaning, disinfection, and adoption of strict biosecurity precautions. In contrast, poor hygiene conditions consisted of no cleaning nor sanitation of the room after the previous occupation by non-experimental pigs. All pigs were housed in individual pens (85 x 265 cm) in concrete floor throughout the experimental period and were fed ad libitum a standard diet formulated to meet or exceed the nutritional requirement of growing pigs (9.47 MJ of net energy/kg, starch 44.2%; fat 3.1%; crude protein 15.3%. They had free access to water. After six weeks of challenge and after an overnight fast, animals (n = 5-9/experimental group) were euthanized by electrical stunning and exsanguination. Immediately after slaughter, samples of subcutaneous dorsal adipose tissue (SCAT) and of longissimus muscle were excised and placed in 37°C Krebs-Ringer-bicarbonate-HEPES buffer for cell isolation (Perruchot et al., 2013).

Adipose cell isolation

Cells were isolated from a sample of fresh dorsal subcutaneous adipose tissue by collagenase digestion as previously described (De clercq et al., 1997; Perruchot et al., 2013). Briefly, fresh

adipose tissue was cut into small pieces and dissociated by enzymatic digestion with collagenase II and XI (800 U/mg; Sigma, St. Quentin Fallavier, France) under shaking in a dry bath for 45 min at 37°C. Then, a centrifugation at 400 g for 10 min was performed to separate floating adipocytes from the pellet of stromal vascular fraction (SVF) cells. After resuspension, SVF cells were successively filtered through a 200-µm and 25-µm nylon membranes (Dutscher, Brumath, France). After isolation, SVF cells were placed in 90% Fetal Calf Serum (FCS) and 10% dimethyl sulfoxide (1–2 million cells per mL) and frozen at − 150 °C.

Muscle cell isolation

Fresh muscle tissue devoid of visible connective tissue was finely chopped and digested for 3 x 20 min in a Ca^{2+}-free medium containing 0.25% trypsin (Invitrogen, Cergy-Pontoise, France), 1.5 mg/mL collagenase II (PAA Laboratoires, Les Mureaux, France), and 0.1% DNAse (Sigma, St. Quentin Fallavier, France) in a shaking water bath at 37°C. After centrifugation at 800 g for 10 min at 4°C, cell pellet was re-suspended in ice-cold proliferation growth medium (PGM) containing DMEM supplemented with 10% FCS (Invitrogen), 10% horse serum (Invitrogen), and Penicillin 6,25 UI/ml -Streptomycin 6,25 µg/ml, (Fisher Scientific, Illkirch, France). Next, cell suspension was successively filtered through a 200-µm and 50-µm Nylon mesh (Dutscher, Brumath, France). After their collection in ice-cold PGM, isolated cells were placed in 90% Fetal Calf Serum (FCS) and 10% dimethyl sulfoxide (1–2 million cells per mL) and frozen at − 150 °C.

Flow cytometry analysis

Individual vials of cryopreserved cells were thawed in a 37°C water bath (2–3 min). After thawing, cells were suspended in 10 mL of FCS (Sigma). After centrifugation at 500 g for 5 min at room temperature (RT), cells were suspended in 10 mL of DPBS (Fisher Scientific, Illkirch, France). Cells were then counted with the TC20™ Automated Cell Counter (Bio-Rad, Marnes-la-Coquette, France). Next, cells were centrifuged at 500 g for 5 min at RT and suspended in 10 mL of FACS buffer. This FACS buffer contained MACSQuant Running Buffer (Miltenyi Biotec, Paris, France) and 0.5% of MACS® BSA Stock Solution (Miltenyi Biotec). Cells were dispensed at 50,000 cells for SCAT and 500,000 cells for muscle in each round bottom tube of 5 mL (Sarstedt, Nümbrecht, Germany). After centrifugation at 500 g for 5 min at RT, cells were

suspended in 100 µL of FACS buffer. Cells were incubated in the dark on ice for 15 min with labeled monoclonal or polyclonal antibodies coupled to different fluorochromes: allophycocyanine (APC), brilliant violet 421 (BV421), fluorescein isothiocyanate (FITC), phycoerythrin (PE), phycoerythrin-vio770 (PE-Vio770) (Table 1). After incubation with antibodies, cells were washed in FACS buffer and centrifuged at 500 g for 5 min at 4°C. Labeled cells were then suspended in 400 µL of FACS buffer. Labeled cells were analyzed using a MACSQuant Analyzer 10 flow cytometer (Miltenyi Biotec), which was calibrated daily. A minimum of 50,000 events was acquired per sample for SCAT and 200,000 events for muscle. Flow cytometry analysis were performed with appropriate isotype matching negative and Flow Minus One (FMO) controls. Data were analyzed using the FlowLogic software (Inivai, Mentone, Victoria, Australia).

Table 1: List of antibodies used for flow cytometry analysis.

Antigen	Antibody	Reference	Supplier
CD34	CD34-PE (clone EP373Y)	ab223930	Abcam
Isotype control	IgG-PE (clone EPR25A)	ab209478	Abcam
CD38	CD38-PE-Vio770 (clone REA 671)	130-119-159	Miltenyi Biotec
Isotype control	IgG1-PE-vio770 (REA 293)	130-113-440	Miltenyi Biotec
CD45	CD45-FITC (clone K252.1E4)	MCA1222F	BIO RAD
Isotype control	IgG1-FITC (clone MCA928F)	MCA928F	BIO RAD
CD56	CD56-BV421 (clone NCAM16.2)	562751	BD Biosciences
Isotype control	(BALB/c)-BV421 IgG2b, κ	562748	BD Biosciences
CD140a	CD140a-APC (clone REA 637)	130-122-942	Miltenyi Biotec
Isotype control	IgG1-APC (REA 293)	130-113-434	Miltenyi Biotec

Statistical analyzes

Statistical analyzes were performed using the GraphPad Prism software (version 8.4.3 (686), San Diego, CA, USA). The data were subjected to the two-way analysis of variance (ANOVA). Hygiene of housing conditions, RFI line and their interactions were considered as fixed effects. Results are presented as mean ± standard error of mean (SEM). Differences were considered statistically significant at $P \leq 0.05$ and were discussed as a trend for $0.05 < P < 0.1$.

Results

Growth performance and body composition of the studied groups

Growth performance (Chatelet et al., 2018) and body composition (Sierżant et al., 2019) of pigs have been previously described for a greater number of experimental pigs. Data for the subset of pigs investigated in the current study are shown in Table 2. First, there was no significant interaction between housing conditions and RFI lines for pig performance and their body composition. Final body weights, average daily gain and backfat weight did not differ between the experimental groups (P > 0.1). Interestingly, irrespective of housing conditions, the relative loin weight was lower in HRFI pigs than in LRFI pigs (P = 0.005). Finally, irrespective of the RFI line, the loin/backfat ratio was higher (P = 0.045) in pigs raised in poor conditions than in pigs raised in good conditions.

	Good		**Poor**		***P-values*[1]**		
	LRFI	**HRFI**	**LRFI**	**HRFI**	**Hyg**	**Line**	**Hyg x Line**
Initial body weight (kg)	27.8 ± 0.7	25.7 ± 1.7	28.2 ± 0.7	25 ± 1.4	0.402	0.495	0.289
Final body weight (kg)	54.6 ± 2.6	51.1 ± 1.9	54.0 ± 3.4	49.8 ± 3.3	0.783	0.174	0.958
Average daily gain (g/day)	626 ± 67	590 ± 59	603 ± 84	564 ± 48	0.750	0.614	0.919
Relative loin weight (%)	27.6 ± 0.3	27.0 ± 0.3	28.4 ± 0.2	27.2 ± 0.5	0.137	**0.005**	0.274
Relative backfat weight (%)	4.9 ± 0.2	5.0 ± 0.1	4.6 ± 0.3	4.6 ± 0.3	0.125	0.903	0.962
Loin/backfat ratio	5.6 ± 0.2	5.5 ± 0.2	6.5 ± 0.5	6.0 ± 0.3	**0.045**	0.322	0.638

Table 2: Growth performance of low (LRFI) and high (HRFI) residual feed intake pigs housed in good or poor hygiene conditions for six weeks.

Values are means ± SEM (n = 5-9 pigs/experimental group). Boldface highlights significant differences (P ≤ 0.05). [1]Probability values for the effect of hygiene conditions (Hyg), genetic lines (Line), and their interaction.

Description of the phenotyping strategy

To identify the cell populations specific and common to SCAT and muscle, we focused our analysis on a panel of five cell surface markers: CD45, CD34, CD38, CD56 and CD140a (Figure 1).

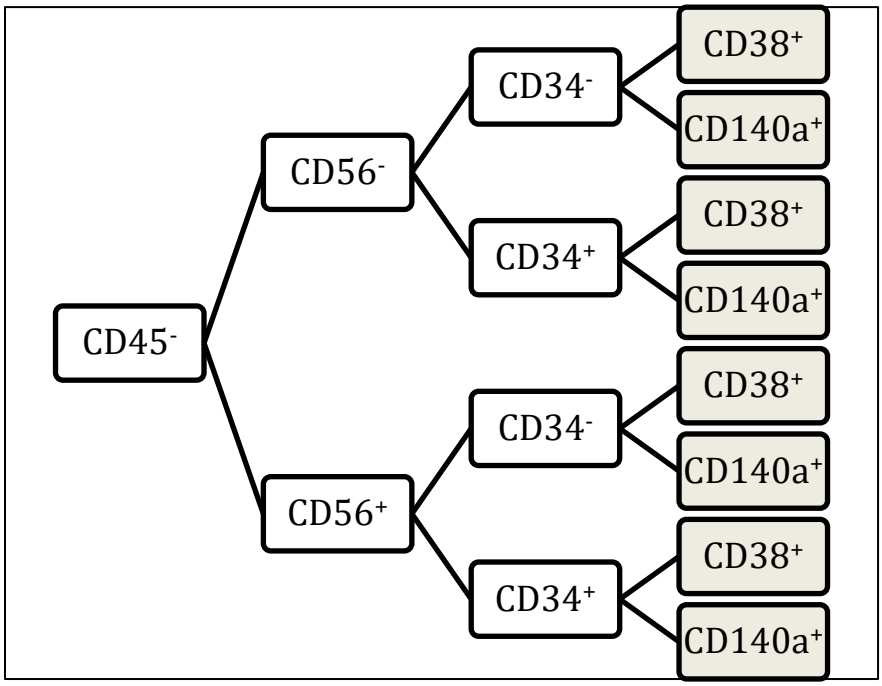

Figure 1: Diagram of the strategy used for cell immunophenotyping by flow cytometry. Adult stem cells were isolated from adipose tissue and skeletal of growing pigs with low and high residual feed intake and housed in good or poor hygiene conditions for six weeks. A common analytical strategy was used for both tissues. However, adipose tissue was phenotyped at the level of triple lineage because of its low quantities of $CD45^-CD56^-CD34^+$ and $CD45^-CD56^+CD34^+$ cells (white squares).

First, gating was performed on living cells that were negative for the CD45 in order to remove hematopoietic cells positive for the CD45 marker. Next, we analyzed the following combinations in SCAT and muscle: $CD45^-CD34^+$, $CD45^-CD38^+$, $CD45^-CD56^-$, $CD45^-CD56^+$, $CD45^-CD140a^+$. Then, we performed a deeper phenotyping for cell populations predominant in both SCAT and muscle, respectively $CD45^-CD56^-$ and $CD45^-CD56^+$ (Figures 2 and 3). Based on the literature, we evaluated within these two cell populations, the expression of the CD34 marker as named: $CD45^-CD56^-CD34^-$, $CD45^-CD56^-CD34^+$, $CD45^-CD56^+CD34^-$ and $CD45^-CD56^+CD34^+$

populations. Given the low proportion (<0.5%) of some cell populations in SCAT, we only analyzed the expression of the CD38 and CD140a cell surface markers in muscle in order to highlight the proportion of the following populations: $CD45^{-}CD56^{-}CD34^{-}CD38^{+}$, $CD45^{-}CD56^{-}CD34^{-}CD140a^{+}$, $CD45^{-}CD56^{-}CD34^{+}CD38^{+}$, $CD45^{-}CD56^{-}CD34^{+}CD140a^{+}$, $CD45^{-}CD56^{+}CD34^{-}CD38^{+}$, $CD45^{-}CD56^{+}CD34^{-}CD140a^{+}$, $CD45^{-}CD56^{+}CD34^{+}CD38^{+}$ and $CD45^{-}CD56^{+}CD34^{+}CD140a^{+}$. Altogether, this gating strategy allowed us to highlight specific and common cell populations in both at SCAT and muscle.

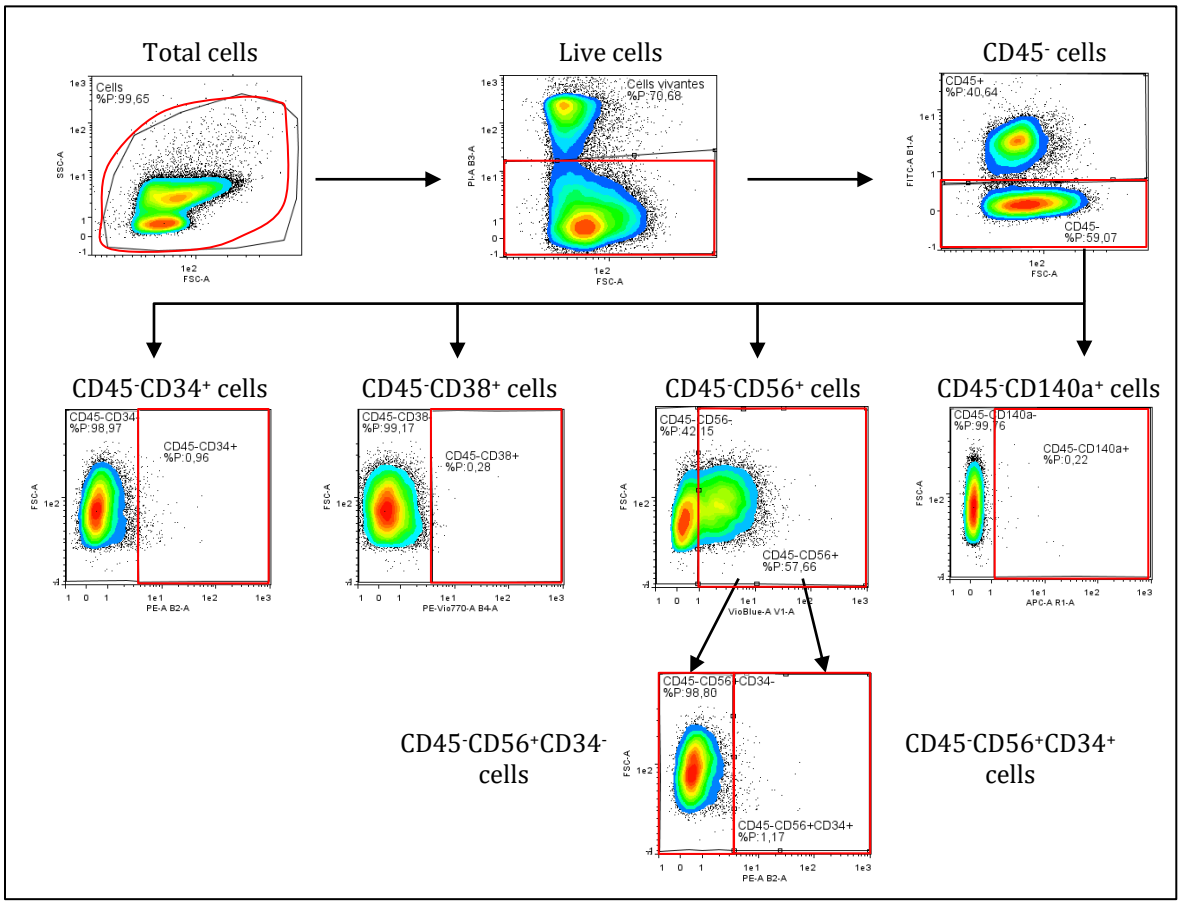

Figure 2: Representative flow cytometry plots illustrating the immunophenotyping strategy used for adult stem cells isolated from adipose subcutaneous tissue of growing pigs. The captions correspond to the cell populations highlighted in red. In addition, the red boxes delimit the positivity threshold of studied cell populations established according to the appropriate isotype and Fluorescence Minus One controls.

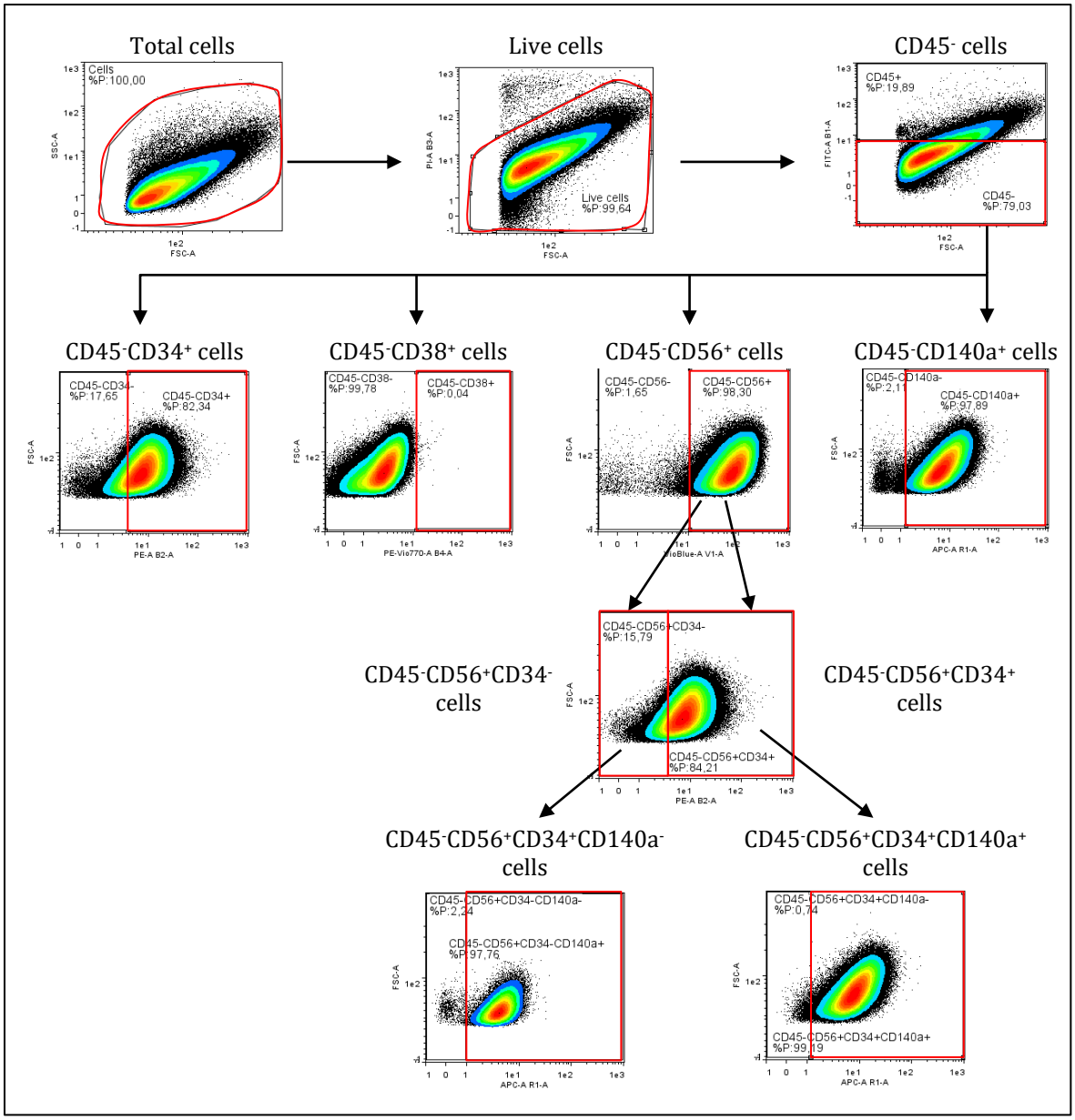

Figure 3: Representative flow cytometry plots illustrating the immunophenotyping strategy used for adult stem cells isolated from skeletal muscle tissue of growing pigs. The captions correspond to the cell populations highlighted in red. In addition, the red boxes delimit the positivity threshold of studied cell populations established according to the appropriate isotype and Fluorescence Minus One controls.

Analysis of cell populations identified in subcutaneous adipose tissue

There was a significant proportion of $CD45^+$ cells (23.1%) in SCAT with no difference between the four experimental groups (Table 3). We next analyzed the occurrence of co-staining for the four cell surface markers CD56, CD34, CD38 and CD140a in order to further delineate the different populations within $CD45^-$ cells dissociated from SCAT. With triple staining, the proportions of the $CD45^-CD56^-CD34^-$ population was higher (+11.7%, P = 0.034) in poor than in good hygiene of housing conditions whatever the RFI line (Figure 4A) whereas the proportion of the $CD45^-CD56^+CD34^-$ population tended to decrease (-17.2%, P = 0.099) in poor hygiene conditions compared with good conditions (Figure 4C). Of note, the other cell populations with double or triple staining, such as $CD45^-CD56^+CD34^+$ cells (Figure 4E) were present in very low proportions (<1%) in SCAT (Table 3).

Table 3: Percentage of adult stem cell populations in subcutaneous adipose tissue of low (LRFI) and high (HRFI) residual feed intake pigs housed in good or poor hygiene conditions for six weeks.

	Good		Poor		*P-values*[1]		
Cell populations	**LRFI**	**HRFI**	**LRFI**	**HRFI**	**Hyg**	**Line**	**Hyg x Line**
$CD45^+$	18.2 ± 7.1	22.7 ± 7.1	26.7 ± 5.1	24.1 ± 5.4	0.434	0.880	0.571
$CD45^-$	81.5 ± 7.1	77.0 ± 7.1	72.7 ± 5.2	75.7 ± 5.4	0.429	0.905	0.558
$CD45^-CD34^+$	0.5 ± 0.2	0.3 ± 0.1	0.4 ± 0.1	0.6 ± 0.1	0.530	0.844	*0.071*
$CD45^-CD38^+$	0.3 ± 0.1	0.2 ± 0.1	0.4 ± 0.1	0.5 ± 0.1	*0.082*	0.779	0.212
$CD45^-CD140a^+$	0.5 ± 0.1	0.1 ± 0.02	0.3 ± 0.1	0.3 ± 0.1	0.607	*0.059*	**0.036**
$CD45^-CD56^-$	27.4 ± 6.0	29.5 ± 5.2	42.4 ± 3.7	36.8 ± 5.1	**0.035**	0.724	0.448
$CD45^-CD56^-CD34^-$	26.8 ± 6.0	29.2 ± 5.2	41.9 ± 3.7	36.4 ± 5.0	**0.034**	0.757	0.442
$CD45^-CD56^-CD34^+$	0.6 ± 0.2	0.2 ± 0.04	0.5 ± 0.2	0.3 ± 0.1	0.746	0.106	0.591
$CD45^-CD56^+$	53.9 ± 12.2	47.3 ± 9.9	30.1 ± 6.2	38.6 ± 10.0	0.101	0.920	0.437
$CD45^-CD56^+CD34^-$	53.7 ± 12.3	47.1 ± 10.0	29.8 ± 6.2	38.3 ± 10.0	0.*099*	0.922	0.439
$CD45^-CD56^+CD34^+$	0.2 ± 0.1	0.2 ± 0.1	0.2 ± 0.1	0.3 ± 0.1	0.230	0.653	0.693

Values are means ± SEM (n = 6-9 pigs/experimental group). Boldface highlights significant differences ($P \leq 0.05$) and italicized characters show a trend ($0.05 < P < 0.10$).

[1]Probability values for the effect of hygiene conditions (Hyg), genetic lines (Line), and their interaction.

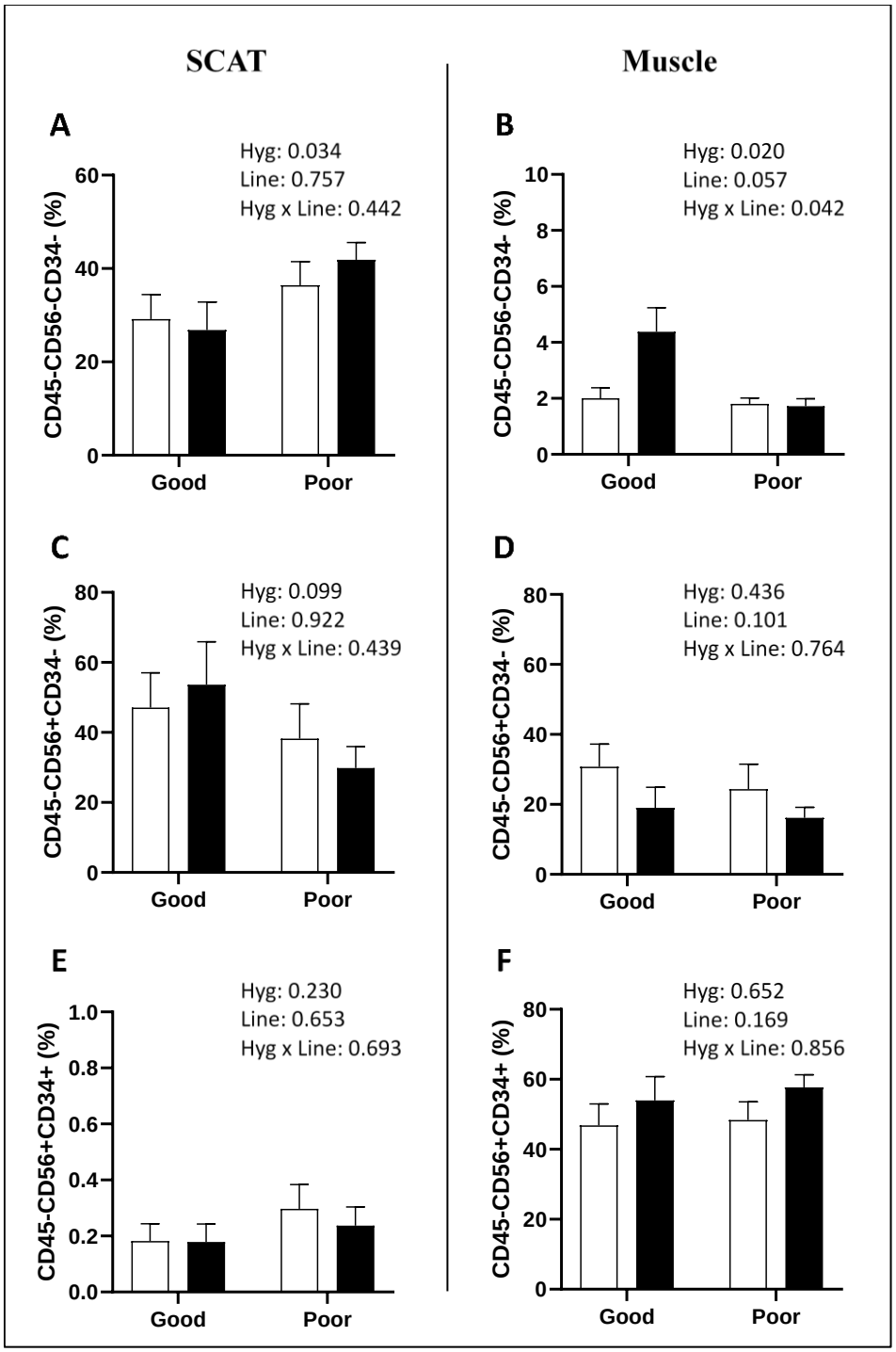

Figure 4: Percentage of adult stem cell populations in subcutaneous adipose tissue (SCAT) and muscle from low (LRFI, white bar) and high (HRFI, black bar) residual feed intake pigs housed in good or poor hygiene conditions. Percentage of cell populations are expressed as means ± SEM (n= 5-9/experimental group).

Analysis of cell populations identified in skeletal muscle

The proportion of $CD45^+$ cells in muscle was in the range of 20-23% (Table 4) and increased (P = 0.020) in pigs housed in poor conditions compared with pigs housed in good conditions for both RFI lines. Next, we analyzed the expression of CD34, CD38, CD140a and CD56 within the $CD45^-$ cell population (Figures 1 and 3). Despite their high proportions (>50%) in muscle, the $CD45^-CD34^+$ and $CD45^-CD140a^+$ cell populations did not differ significantly between the four groups. Furthermore, there was an interaction for the $CD45^-CD56^-$ cell population (P = 0.011). Interestingly, within this $CD45^-CD56^-$ cell population, we analyzed the expressions of CD34 and CD140a. We also observed a significant interaction for the $CD45^-CD56^-CD34^-$ cell population (P = 0.042) (Figure 4B). For the $CD45^-CD56^-CD34^-CD140a^+$ cells, their proportion tended to decrease (P = 0.072) in poor compared with good hygiene conditions. Moreover, within the $CD45^-CD56^+$ cell population, the proportion of $CD45^-CD56^+CD34^-$ cells tended to be higher in HRFI pigs (+10%, P = 0.101) than in LRFI pigs (Figure 4D). Concerning the $CD45^-CD56^+CD34^+$ cells, their proportions do not vary whatever the genetic line or the housing conditions (Figure 4F). Finally, by adding the CD140a marker in the analysis, we highlighted that, irrespective of the genetic line, the $CD45^-CD56^+CD34^+CD140a^+$ cells increased (+ 11.3%) in poor conditions (P = 0.052) compared with good conditions (Table 4).

Table 4: Percentage of cell populations in muscle of low (LRFI) and high (HRFI) residual feed intake pigs housed in good or poor hygiene conditions for six weeks.

	Good		Poor		*P-values*[1]		
Cell populations	**LRFI**	**HRFI**	**LRFI**	**HRFI**	**Hyg**	**Line**	**Hyg x Line**
$CD45^+$	20.1 ± 1.5	18.6 ± 1.4	23.0 ± 0.9	23.1 ± 1.9	**0.020**	0.620	0.560
$CD45^-$	79.0 ± 1.5	80.6 ± 1.4	76.4 ± 1.0	76.1 ± 2.1	**0.030**	0.678	0.552
$CD45^-CD34^+$	53.3 ± 7.3	47.9 ± 5.8	58.2 ± 3.7	49.5 ± 4.6	0.583	0.242	0.790
$CD45^-CD38^+$	0.1 ± 0.02	0.1 ± 0.02	0.1 ± 0.03	0.3 ± 0.1	0.121	0.447	*0.091*
$CD45^-CD140a^+$	55.4 ± 9.7	67.2 ± 6.4	69.1 ± 3.5	67.2 ± 0.8	0.327	0.478	0.327
$CD45^-CD56^-$	5.6 ± 0.9	2.6 ± 0.5	2.3 ± 0.3	2.8 ± 0.5	**0.024**	*0.073*	**0.011**
$CD45^-CD56^-CD34^-$	4.4 ± 0.9	2.0 ± 0.4	1.7 ± 0.3	1.8 ± 0.2	**0.020**	*0.057*	**0.042**
$CD45^-CD56^-CD34^-CD140a^+$	1.8 ± 0.7	1.0 ± 0.3	0.5 ± 0.1	0.5 ± 0.2	*0.072*	0.362	0.351
$CD45^-CD56^-CD34^+$	1.2 ± 0.5	0.6 ± 0.2	0.5 ± 0.1	1.0 ± 0.4	0.735	0.855	0.115
$CD45^-CD56^-CD34^+CD140a^+$	0.6 ± 0.4	0.3 ± 0.2	0.2 ± 0.04	0.7 ± 0.4	0.943	0.904	0.210
$CD45^-CD56^+$	73.3 ± 1.4	77.9 ± 1.5	74.1 ± 1.1	73.2 ± 2.4	0.224	0.245	*0.099*
$CD45^-CD56^+CD34^-$	19.0 ± 5.9	30.8 ± 6.5	16.2 ± 3.0	24.4 ± 7.1	0.436	0.101	0.764
$CD45^-CD56^+CD34^-CD140a^+$	17.9 ± 5.9	29.3 ± 6.4	15.6 ± 2.9	23.6 ± 7.0	0.499	0.108	0.771
$CD45^-CD56^+CD34^+$	54.0 ± 6.8	46.8 ± 6.1	57.7 ± 3.6	48.4 ± 5.2	0.652	0.169	0.856
$CD45^-CD56^+CD34^+CD140a^+$	36.3 ± 5.5	37.7 ± 5.4	52.7 ± 3.3	43.0 ± 6.2	**0.052**	0.433	0.298

Values are means ± SEM (n = 5-7 pigs/experimental group). Boldface highlights significant differences ($P \leq 0.05$) and italicized character shows a trend ($0.05 < P < 0.10$). [1]Probability values for the effect of hygiene conditions (Hyg), genetic lines (Line), and their interaction. The proportions of $CD45^-CD56^-CD34^-CD38^+$, $CD45^-CD56^-CD34^+CD38^+$, $CD45^-CD56^+CD34^-CD38^+$ and $CD45^-CD56^+CD34^+CD38^+$ populations were below 0.2%.

Discussion

The current study was conducted to determine whether the exposure of growing HRFI and LRFI pigs to poor hygiene of housing conditions impact the proportions of adult stem cell populations resident in muscle and adipose tissue, two tissues of importance for body composition and meat quality traits. Whatever the species considered, the characterization of adult stem cells in muscle and adipose tissue is fairly recent and is conventionally carried out using flow cytometry which is based on the use of antibodies directed against cell surface proteins (Bourin et al., 2013; Relaix et al., 2021). For the current cell immunophenotyping, we have used five well-known cell surface markers (CD45, CD56, CD34, CD38 and CD140a) to identify specific and common $CD45^-$ stem cell populations in both tissues. Moreover, we analyzed for the first time $CD45^+$ cells, a population of hematopoietic cells (Han et al., 2021) known to be increased after injury in muscle (Wosczyna et al., 2019). Our major finding is that the proportions of several cell populations were affected by hygiene of housing conditions in a tissue-dependent manner in pigs of both RFI lines.

The lower growth performance of pigs housed in poor hygiene conditions compared with pigs housed in good hygiene conditions described with a greater number of animals than in the current subset of animals (Chatelet et al., 2018) and the increase in their loin/backfat ratio was associated with changes in the relative proportions of some cell populations in adipose tissue and skeletal muscle. Even though health and growth performance of the most feed efficient LRFI pigs was less impaired by poor hygiene conditions (Chatelet et al., 2018), our findings at the cellular levels do not support a significant relationship between MSC and the better coping ability of LRFI compared with HRFI pigs. In the current study, there was only a significant interaction for the $CD45^-CD56^-CD34^-$ population in muscle, a relatively small population. For the other cell populations, there was no significant difference between the two lines. This differs slightly from a previous study showing some differences in some cell populations between the two RFI lines (Perruchot et al., 2020).

The current study clearly shows the presence of a relative high proportion of $CD45^+$ cells suggesting the presence of hematopoietic cells in both muscle and adipose tissue as shown in previous studies (Cousin et al., 2016; Wosczyna et al., 2019). Our study also indicates that the

proportion of $CD45^+$ cells in muscle increased with the exposure of pigs to poor hygiene of housing conditions. This increase may be associated with the inflammation induced by the poor hygiene of housing conditions. The stimulation of the immune system of pigs housed in these conditions was assessed by an increase in plasma haptoglobin concentrations, an acute phase protein (APP), and in white blood cell and granulocyte counts three weeks after the beginning of the challenge (Chatelet et al., 2018). The origin of this increase in $CD45^+$ cells remains unknown. It could result from an increase in $CD45^+$ resident cells in skeletal muscle. Alternatively, it could come from other tissues as reported for MSCs in a recent review (Girousse et al., 2021). A study based on the investigation of MSCs from adipose tissue and peripheral blood of Iberian pigs supports an in vitro migration ability of these cells (Calle et al., 2018). Resident MSCs could circulate toward distant inflamed tissues to allow their repair/regeneration. Hence, the observed increase in $CD45^+$ cells may be associated with changes in local secretory environment and may affect properties of other muscle cells and affect subsequent growth.

As described previously (Bourin et al., 2013; Girousse et al., 2021; Relaix et al., 2021) including in our previous work (Perruchot et al., 2013, 2020), there was a diversity of adult stem cell populations in skeletal muscle and adipose tissue of the different groups of animals. We confirmed that $CD45^-CD56^+$ cells are predominant in both muscle and adipose tissue as observed before (Perruchot et al., 2013). In porcine muscle, these cells are mainly myogenic (Perruchot et al., 2013). In human and pig, $CD45^-CD56^+CD34^-$ were identified as satellite cells (Wilschut et al., 2008; Pisani et al., 2010b; Lewis et al., 2014). In adipose tissue, the presence of $CD45^-CD56^+$ is much less documented. We further highlighted a higher cell diversity in muscle than in adipose tissue. There was especially a high proportion of $CD34^+$ and $CD140a^+$ cells within the $CD45^-$ cells. In pigs, $CD45^-CD34^+$ cells have been previously identified in muscle as $PW1^+$ interstitial cells (PICs) with an ability to differentiate into skeletal myoblast/myotubes, smooth muscle, and endothelial cells (Lewis et al., 2014). The $CD45^-CD140a^+$ cells were also located in the interstitial space of muscle. In normal skeletal muscle and damaged skeletal muscle, $CD140a^+$ cells exhibit fibrogenic and adipogenic potentials and were named fibro/adipogenic progenitors (FAPs; Uezumi et al., 2010, 2014; Uezumi, Ikemoto-Uezumi & Tsuchida, 2014). In muscle of growing pigs, we have shown that $CD56^+CD140a^+$ and $CD56^+CD140a^-$ were mainly myogenic (Perruchot et al., 2020). Muscle stem cell heterogeneity has been described at several

other levels, including transcription factor, clonal capacity, metabolism, age dependent myogenic potential, and recently functional response to environmental stress (Cho & Doles, 2019; Relaix et al., 2021). This multitude of resident stem cells contributes likely together to the remarkable regeneration capacities of muscle after injury (Mackey et al., 2017; Relaix et al., 2021). We also included the CD38 marker in our analysis to determine whether it could be used to identify committed preadipocytes as described in mice (Carrière et al., 2017). However, the very low number of cells expressing this marker does not support these findings. The discrepancy between the two studies may be related to the fact that the CD38 marker was detected in cells from abdominal adipose tissue, an adipose depot exhibiting a greater expression of CD38 than in SCAT.

We highlighted for the first time that the environment influence MSCs in growing pigs. The $CD45^{-}CD56^{+}CD34^{+}CD140a^{+}$ cell population, one of the abundant populations in muscle, increased clearly after an exposure of pigs to poor hygiene of housing conditions. We previously found that $CD56^{+}CD140a^{+}$cells were myogenic (Perruchot et al., 2020). With the use of the CD34 and CD56 markers, $CD34^{+}$ cells derived from human muscle were described to be myo-adipogenic bipotent progenitors (Pisani et al., 2010a,b). Thus, we can hypothesize that these cells exhibit myo/adipogenic potentials. It is interesting to note that $CD56^{+}CD140a^{+}$ cells were also affected by a high-fat/high-fiber diet in porcine muscle (Perruchot et al., 2020). This suggests that these cells may respond to changes of different factors. The current study further indicates that there was an interaction between hygiene conditions and RFI lines for $CD45^{-}CD56^{-}$ cells including $CD45^{-}CD56^{-}CD34^{-}$ cells representing a low proportion of cells in muscle. There was a decrease of cells in poor hygiene condition only in LRFI pigs. These cells may be perivascular cells as described for $CD146^{+}CD34^{-}CD45^{-}CD56^{-}$ cells in multiple human tissues including muscle (Crisan et al., 2008; Shenoy & Bose, 2018). These cells may have a myo-adipogenic potential. Indeed, we have shown that $CD56^{-}CD140a^{+}$ cells in porcine muscle exhibited both adipogenic and myogenic potentials (Perruchot et al., 2020). Moreover, Shenoy & Bose (2018) showed that pericytes from mouse liver were able to regenerate muscle fibers in muscle of dystrophic (Mdx) mice. In addition, it has been shown in skeletal muscle of obese humans that the $CD56^{-}$ cell fraction gives rise to white adipocytes (Laurens et al., 2016). Other studies have reported the existence of adipogenic progenitors, not expressing the CD56 marker, in skeletal

muscle MSCs derived from lean individuals in humans (Vauchez et al., 2009; Lecourt et al., 2010). Altogether, available data obtained in pigs and humans are consistent with a myo-adipogenic potential of the $CD45^-CD56^-CD34^-$ cells.

Adipose tissue cell composition is less complex than skeletal muscle with two abundant $CD45^-$ cell populations, the $CD45^-CD56^-CD34^-$ and the $CD45^-CD56^+CD34^-$ populations. The proportion of $CD45^-CD56^-CD34^-$ cells increased whereas the proportion of $CD45^-CD56^+CD34^-$ tended to decrease in degraded hygiene conditions. The presence of $CD45^-CD56^+CD34^-$ cells in adipose tissue is poorly documented in the literature. In two previous studies, we provided evidence that $CD56^+$ cells from subcutaneous adipose tissue were less adipogenic than $CD56^-$ cells (Perruchot et al., 2013, 2020). Some groups did not find any $CD56^+$ cells in adipose tissue, while others attribute the expression of CD56 to natural killer cells ($CD45^+$). Overall, there is no consensus on the expression of the CD56 marker in adipose tissue within the $CD45^-$ cells. Moreover, the increase in $CD45^-CD56^-CD34^-$ cells in poor hygiene condition may increase later adipose tissue growth in this group of pigs. Another possible hypothesis would be that the poor hygiene condition of pig housing may lead to a delay in the development of adipose tissue, and therefore the pool of $CD45^-CD56^-CD34^-$ cells, potential adipocyte precursors, would be less used in these animals compared with those housed in good condition. Altogether, these findings are consistent with studies demonstrating that the composition of the MSC compartment of adipose tissue and skeletal muscle can be affected by several factors including nutritional factors (Perruchot et al., 2020) during growth.

Conclusions

The findings gathered in this study clearly show that the relative proportions of hematopoietic and of some MSC populations were affected by hygiene of housing conditions in a tissue-dependent manner in pigs of both RFI lines. It further indicates that these cell populations may play a significant role in adipose tissue and skeletal muscle homeostasis and can be targeted to modulate growth and body composition in growing animals. Further investigations will be needed to get a better understanding of cell interactions in both tissues.

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Data accessibility

Data are available online: https://doi.org/10.15454/IXXVBB

Supplementary material

There is no supplementary material.

Conflict of interest disclosure

The authors of this preprint declare that they have no financial conflict of interest with the content of this article

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