Response of adult stem cell populations to a high-fat/high-fiber diet in skeletal muscle and adipose tissue of growing pigs divergently selected for feed efficiency

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Abstract:

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Purpose The control of body composition by dietary nutrients is of the upmost importance for both human and animal physiology. Adult stem cells (aSC) may represent a relevant level of tissue adaptation. The purpose of this study was to determine the impact of macronutrient composition on aSC populations isolated from adipose tissue or muscle in growing pigs.

Methods Pigs from two lines divergently selected for feed efficiency were fed ad libitum either a low-fat high-starch (LF) diet or a high-fat high-fiber (HF) diet (n = 6 per line and diet) from 74 to 132 days of age. Stroma-vascular cells were isolated from adipose tissue and muscle and characterized with cell surface markers.

Results Pigs fed the HF diet exhibited a reduced adiposity (P < 0.001) compared with pigs fed the LF diet whatever the line. In the four groups, CD90 and PDGFRa markers were predominantly expressed in adipose cells, whereas CD90 and CD56 markers were highly expressed in muscle cells. In adipose tissue, the proportion of CD56+/PDGFRa+ cells, and the proportion of CD90+/PDGFRa+ were lower (P < 0.05) in HF pigs than in LF pigs. On the opposite, in muscle, these proportions were higher (P < 0.001) in HF pigs.

Conclusion This study indicates that dietary nutrients affected the relative proportions of aSC populations in pigs, with opposite effects on CD90+/PDGFRa+, CD56+/PDGFRa+ cell populations between muscle and adipose tissue. Therefore, the control of these cells may be a strategy to modulate body composition.

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Keywords Adult stem cells. Feed efficiency. Growth. Adipose tissue. Skeletal muscle

Introduction

The control of body composition by nutrition is a topic of importance for both humans and farm animals. In humans, the increase in the prevalence of obesity throughout the world (OECD obesity update 2017; https://www.oecd.org/els/health-systems/Obesity-Update-2017.pdf) highlights the need to prevent and/or treat this pathology that is associated with an increased adiposity and a decrease in muscle mass [1]. In animals, the lean-to-fat ratio and the intramuscular fat content are of special interest for the economy of production and meat quality traits [2]. The development of adipose tissue and skeletal muscle is basically triggered by variations in the number and size of constitutive cells. These cells are recruited from various populations of multipotent mesenchymal stem cells (MSC) resident in tissues, which are also named adult stem cells (aSC) [3]. They are characterized by their ability to self-renew and to differentiate into cell types of different lineages and by the expression of cell surface markers [21].

In adults, it has been shown that different degrees of adiposity mass are associated with changes in the biology of aSC in lean and fat tissues [4]. For instance, aSC from adipose tissue of obese subjects have impaired differentiation and migration properties compared with aSC from non-obese patients [5,6]. Other studies have described the responses of aSC from skeletal muscle to dietary changes, such as short-term calorie restriction or dietary lipid composition [7,8]. We hypothesized that the responses of aSC populations to genetics and nutritional challenges may differ between tissues.

Both dietary calories and the balance of nutrients in diets influence lean and fat mass. For instance, the increase in adiposity that is generally induced by a chronic intake of high-fat diets [9,10] can be attenuated by dietary fibers [9,10]. Recently, we used an experimental strategy combining genetics and nutrition to induce marked differences in adiposity in growing pigs and study transcriptomic and metabolic changes in adipose tissues [14, 15]. This species has strong anatomical and physiological similarities with humans [9,10], and the findings of differences between rodent and human genome [11] reinforce the need to investigate other experimental animals than rodents for metabolic research [12]. The present study was undertaken to determine the impacts of genetic propensity for feed efficiency (net use of nutrients by tissues) and of contrasted dietary energy source (starch vs. fat and fiber) on the relative proportion of aSC populations in adipose tissue and muscle. Associations between different sub-populations and changes in body compositions were then determined.

Materials and methods

Ethics statement

The care and use of pigs were performed in compliance with the European Union legislation (directive 2010/63/EU). The protocol was approved by the local Ethics Committee in Animal Experiment of Rennes, France (Comité Rennais d'Ethique en matière d'Expérimentation Animale, CREEA, http://ethique.ipbs.fr/creeapresent.html; agreement N°R-2012-07). All animals were reared and killed in compliance with national regulations, and according to procedures approved by the French Veterinary Services.

Animals, diets and slaughtering

The present study was part of a larger work whose results related to growth performance, body composition and tissue metabolic pathways have been published [11,12]. In brief, a total of 24 pigs (purebred French Large White barrows) from two genetic lines divergently selected over eight generations of residual feed intake (RFI), a measure of feed efficiency corresponding to the net use of nutrients by tissues [13], were considered as a representative subset (n = 6 per line and per diet) of the full design. From 74 days of age to 132 days of age, pigs were housed individually (full slatted floor) allowing individual feed intake recording (offers minus refusals). Within each line, pigs were randomly assigned to one of the two dietary groups, and fed ad libitum either a low-fat, low-fiber (LF) diet or a high-fat, high-fiber (HF) diet. The diets were formulated in two formulas distributed during the growing and finishing periods, respectively, and for which nutritional composition (protein content and energy values) followed recommendations for optimal growth during these two periods. The HF and LF diets included similar crude protein contents (17% and 13% for growing and finishing formulas, respectively) and digestible essential amino acids (lysine, methionine and threonine). Starch derived from cereal grains (wheat and barley) in the LF diet was partially replaced by rapeseed and soybean oils in the HF diet and crushed wheat straw (insoluble fiber) was included as a diluent of dietary energy in this diet, so that LF and HF diets were formulated on an isocaloric basis (12.9 MJ/kg metabolizable energy). This strategy resulted in large variations between HF and LF diets in their nutritional composition (as fed basis): respectively, 33.5% vs. 45.5% of starch, 7.2% vs. 2.2% of fat, 18% vs. 12.1% of neutral-detergent fiber and 8.2% vs. 3.6% of acid-detergent fiber. Detailed diet composition was given in Table 1. Pigs were fed experimental diets during 58.5 ± 0.5 days. All pigs were killed at the same age (132.5 ± 0.5 days) at an average BW of 75.3 ± 1.2 kg. They were euthanized by electronarcosis followed by jugular exsanguination 2 h after their first morning meal, at the INRA experimental facilities (35590 Saint-Gilles, France).

Tissue sampling

Samples of subcutaneous dorsal adipose tissue (SCAT) and of skeletal muscle (longissimus lumborum) were rapidly excised. Slices of adipose tissue (15 g) were immediately placed in 37°C Krebs-Ringer bicarbonate buffer, whereas slices of muscle (15 g) were placed in ice-cold Dulbecco's Phosphate Buffer Saline (DPBS, Dutscher, Brumath, France) containing 1% glucose (D-Glucose; Sigma, Saint-Quentin Fallavier, France), and further used for cell isolation. Other SCAT and muscle (oriented according to myofiber longitudinal axis) samples (0.5 x 0.5 x 1 cm) were fixed with pins on flat sticks, frozen in 2-methylbutane (isopentane) chilled with liquid nitrogen, and stored at -70°C until histological procedures.

Immunohistochemistry

Thick transverse cross-sections (8 μm) of tissues were obtained using a cryostat (2800 FrigocutN, Reichert Jung, Heidelberg, Germany) equilibrated at -30 and -20°C for adipose tissue and muscle, respectively. Sections were mounted on silane-coated slides for enhanced adhesion. Immunocyto-localization of PDGFRα marker was carried out using a standard procedure. Briefly, after incubation in 2% bovine serum albumin (BSA; Sigma, Saint-Quentin Fallavier, France) to prevent non-specific binding, sections were incubated in PBS/0.1% BSA for 45 min at room temperature with the following primary mouse antibodies: anti-PDGFRα and anti-laminin complex (Interchim, Montluçon, France). Nuclei were counterstained with 4'-6-diamidino-2-phenylindole (DAPI; Dako, Trappes, France). No significant staining was detected in slides incubated with control serum and/or goat IgG in the absence of the primary antibody. A Nikon DS-Ri1 epifluorescence microscope was used to acquire digital images using an Eclipse E400 digital camera and NIS–Elements software version 3.0 (Nikon). Qualitative and quantitative analyses of stained cells were performed using a self-developed plugin for ImageJ (ImageJ 1.43, National Institutes of Health, Bethesda, MD, USA).

Cells isolation

Cells were isolated from adipose tissue by collagenase digestion according to De Clercq et al. [14]. Adipose tissue was minced and digested in a 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid phosphate buffer (HEPES, 2 mL/g of tissue) containing 2% BSA and 2 mg/mL collagenase II and XI (800 U/mg; Sigma) in a shaking water bath for 45 min at 37°C. A fivefold excess of collagenase buffer free was added before cells were centrifuged at $400 \times g$ for 10 min to separate floating adipocytes from the pellet of stroma vascular (SV) cells. After re-suspension in Dulbecco's Modified Eagle Medium (DMEM), SV cells were successively filtered

133 through a 200- and 25-µm nylon mesh (Nytex, Dutscher Brumath, France). Cells were isolated from skeletal

134 muscle according to Perruchot et al. [15]. Muscle was digested for one hour in a water bath in a mixture of

135 trypsin 0.25% (Invitrogen, Cergy Pontoise, France)/Collagenase de type II 1.5 mg (PAA Les Mureaux,

136 France)/DNAse 0.1% Sigma Saint-Quentin Fallavier, France). After centrifugation, cell pellet was suspended in

137 DMEM and filtered through a 200 µm Nylon membrane and then a 50 µm Nylon membrane (Nytex, Dutscher

138 Brumath, France). The SV cell sub-fractions isolated from adipose tissue or skeletal muscle were placed in 90%

139 Fetal Calf Serum (FCS) and 10% dimethyl sulfoxide (1 to 2 million cells per mL) and frozen at -150°C.

140

141 **Flow cytometry**

142 Viable cells in SV sub-fractions were thawed and counted with the Vi-Cell^{TM} XR analyzer using the trypan-blue

143 exclusion test (Beckman Coulter, Paris, France). Cells with viability greater than 90% were further analyzed for

144 cell surface markers using human or porcine cross-reactive antibodies (Table 1). About 800, 000 cells were

145 placed in PBS containing 0.5% FCS and incubated with labeled monoclonal antibodies (mAb) coupled to

146 different fluorochromes [Phycoerythrin-cyanine 7 (PE-Cy7), R-Phycoerythrin (RPE), phycoerythrin (PE),

147 fluorescein isothiocyanate (FITC), Allophycocyanine (APC)] or with a non-labeled anti-PDGFRα antibody

148 which was detected using an appropriate FITC-conjugated secondary antibody. All data were compared to

149 isotype-matched negative controls. Labeled cells were then analyzed using a MACSQuant® flow cytometer and

150 software (Miltenyi Biotec, Paris, France). A minimum of 20,000 events was acquired for each sample.

151

152 *Cell selection using magnetic microbeads*

153 Frozen cells were thawed and were first counted using a Malassez hemocytometer. Cells were then sorted using

154 antibodies against CD56, PDGFRα, and CD45 cell surface markers using magnetic activated cell sorting (MACS

155 ® technology) (Miltenyi Biotec, Bergisch Gladbach, Germany). The cell suspension was first treated with an anti-

156 pig CD45 mouse monoclonal antibody (Table 2). After antibody binding, CD45 positive cells were removed

157 through indirect anti-FITC IgG microbead sorting (Miltenyi Biotec), leaving the CD45- fraction. From this CD45-

158 fraction, the PDGFRα+ or CD56+ cells were enriched through incubation with a polyclonal anti-PDGFRα

159 antibody followed by incubation with an appropriate PE-conjugated secondary antibody or with a monoclonal

160 anti-pig CD56 antibody (Table 2) followed by anti-PE IgG microbead sorting (Miltenyi Biotec) or anti-APC IgG

161 microbead sorting, respectively. Cells were purified using MACS. The purity of the preparation was assessed by

162 flow cytometry.

163

Cell culture

165 Cells isolated from adipose tissue and muscle were thawed and counted with the Vi-Cell[TM] XR analyzer using

166 the trypan-blue exclusion test (Beckman Coulter). After centrifugation, some cells were maintained in the

167 control medium (DMEM supplemented with 10% FCS, 0.25 µg/mL amphotericin D and 20 µg/mL gentamicin)

168 for 9 days to confirm the absence of spontaneous differentiation. Other cells were placed in a medium allowing

169 adipogenic or myogenic differentiation. For both conditions, cells were seeded at a density of 7 x 10^4 cells/cm² in

170 six-well plates (9.6 cm²/plate) coated with growth factor reduced Matrigel (1/50 v/v, BD Biosciences, Le Pont de

171 Claix, France) and grown at 37°C under 95% air and 5% CO_2.

172 Adipogenic and myogenic differentiations were assessed as previously described [16]. In adipogenic condition,

173 on the first day of culture (day 1), cells were placed in a medium with 10% swine serum. On day 3 of culture, the

174 medium was switched to 2.5% swine serum. After 5 days of culture, cells achieved 80% confluence and were

175 induced to differentiate in DMEM Glutamax High Glucose supplemented with 25 µM IBMX (3-Isobutyl-1-

176 methylxanthine), 20 µg/mL gentamicin, 0.25 µg/mL fungizone, 8 nM insulin, 1 µM dexamethasone, 10 µM

177 troglitazone, 0.2 nM Triiodothyronine (T3), 10 µg/mL transferrin and 0.1 nM ascorbic acid. Lipid accumulation

178 in cells was estimated using ImageJ (3 wells per animal and 3 random fields per well containing approximately

179 900 cells). Results were expressed as the surface of lipid droplets divided by the total area of cells in the same

180 field. In myogenic condition, the fusion index was estimated at 3 wells per animal and 3 random fields per well

181 and expressed as the number of DAPI stained nuclei inside myotubes that were stained with MF-20 antibody

182 (Developmental Studies Hybridoma Bank, Iowa) when divided by the total number of nuclei in the same field. A

183 Nikon DS-Ri1 epifluorescence microscope was used as previously described for immunohistochemistry.

184

Statistical analysis

186 Data were analyzed by ANOVA considering the effects of genetic lines (RFI-/RFI+), diet (HF/LF) and their

187 interaction (line x diet) using the R software version 3.0.2 (R Development Core Team, 2008) followed by

188 Turkey's multiple comparison test. Differences were considered significant at $p \leq 0.05$, whereas $0.05 < p < 0.10$

189 was considered as a trend.

190

Results

Body composition of investigated pigs

Growth performance and body composition of pigs have been previously described for the full experimental design [12]. Data are summarized for the subset of pigs investigated in the current study (Table 3). Importantly, there was no significant interaction between diet and line on performance and body composition. Irrespective of RFI line, pigs fed a high-fat high-fiber (HF) diet ate less ($P = 0.01$) than pigs fed the low-fat low-fiber starch-based (LF) during the experimental period. Compared with the LF diet, the HF diet reduced the backfat proportion in the carcass in both lines ($P < 0.001$) and increased the proportion of lean mass ($P < 0.05$). The loin/backfat ratio was increased in both lines of pigs receiving the HF diet compared with pigs receiving the LF diet (+25% on average, $P < 0.001$).

In situ localization of PDGFRα in adipose tissue and skeletal muscle

In SCAT, immunohistochemical staining with antibodies against PDGFRα and laminin revealed that PDGFRα+ cells were mostly present in the stroma in close vicinity to mature adipocytes and capillaries (Fig. 1A). In muscle, PDGFRα+ cells were detected in the perimysium and the interstitial space (endomysium) between muscle fibers but not in the satellite position (Fig. 1B).

Expression of individual cell surface markers in SV cells from SCAT and skeletal muscle

Flow cytometry analysis revealed significant expressions of CD34, CD56, CD90 and PDGFRα markers on the surface of SCAT cells (Fig. 2A). Whereas the proportion of cells expressing the CD34 marker was less than 2% and did not differ between groups, proportions of cells expressing CD56+, CD90+ and PDGFRα+ markers were highly dependent on the groups. The proportion of CD56+ cells was similar between pig lines, but it was lower in HF pigs than in LF pigs (-40% on average, $P < 0.001$). The proportion of CD90+ cells was lower in RFI+ than in RFI- pigs, but it was not affected by the diet. For PDGFRα+ cells, there was a lie x diet interaction ($P = 0.001$); indeed, the proportion of PDGFRα+ cells was lower in RFI- pigs fed HF diet than in RFI- pigs fed LF diet ($P < 0.05$), but was not affected by diet in RFI+ pigs.

In skeletal muscle, CD56+ and CD90+ cells were the most represented subtypes, with 22-25% and 17-28% of the cells, respectively (Fig. 2B). The proportion of CD56+ cells did not differ between groups. The proportion

218 of CD34+ cells was affected by diet and line with a lower proportion of cells in HF than in LF pigs (-25% on

219 average, P < 0.01) and a greater proportion of cells in RFI- than in RFI+ pigs (P < 0.001). The proportion of

220 CD90+ cells was not affected by the diet and was lower in RFI- pigs than in RFI+ pigs (P < 0.001). Compared

221 with the LF diet, the HF diet increased the proportion of PDGFRα+ in both lines (P < 0.001); it also tended to be

222 lower in RFI+ pigs than in RFI- pigs (P < 0.07)..

223

Expression of combined cell surface markers in SV cells from SCAT and skeletal muscle

225   In SCAT (Fig. 3A), the HF diet reduced the proportion of CD90+/PDGFRα+ cells (P < 0.05) compared with

226 the LF diet. (suppl. Table 1). On the opposite, in muscle (Fig. 3B), the proportions of CD90+/PDGFRα+ cells

227 were increased (P < 0.001) by the HF diet compared with the LF diet. In SCAT, the proportion of

228 CD56+/PDGFRα- cells was lower in HF pigs than in LF pigs (P < 0.05) whereas in muscle, the proportion of

229 CD56+/PDGFRα+ cells was higher (P < 0.001) in HF than in LF pigs.

230

Relationships between the percentage of cell populations in SCAT and skeletal muscle and tissue weights

233 Positive correlations (r > 0.50; P < 0.05) were observed between SCAT relative proportion of the body, used as a

234 surrogate of adiposity, and proportions of CD34-/PDGFRα+, CD56+/PDGFRα+ and CD90+/PDGFRα+ cells in

235 SCAR, whereas a negative correlation (P < 0.05) was observed between these cells populations in muscle and

236 the percentage of the loin (Table 4). There was also a positive correlation between loin mass and proportion of

237 CD34+/PDGFRα- cells (P < 0.001) in muscle.

238

Adipogenic and myogenic differentiation potentials of sorted cells

240 Cells selected using the combination of CD56 and PDGFRα markers (Fig. 4) were further analyzed for

241 developmental features in adipogenic or myogenic differentiation media cells isolated from SCAT and placed in

242 myogenic conditions did not exhibit any significant expression of MF-20 as indicative of myotubes fusion. In

243 adipogenic conditions, these cells differentiated into multilocular adipocytes (Fig. 5A); however, the percentage

244 of differentiation reached 72.5 ± 7% for CD56-/PDGFRα+, 26.5 ± 7% for CD56+/PDGFRα+ and was below

245 5% for CD56+/PDGFRα− cells, respectively.

246   In muscle, all cells formed long multinucleated myotubes when cultured in a myogenic medium. The fusion

247   index reached 61 ± 5%, 72 ± 6% and 21 ± 4%, for CD56+/PDGFRα+, CD56+/PDGFRα− α and CD56-

248   /PDGFRα+, respectively. In adipogenic conditions, CD56-/PDGFRα+ cells were filled with lipid droplets and

249   42 ± 4% of these cells differentiated into mature multilocular adipocytes (Fig. 5B). The rate of differentiation

250   into adipocytes was lower than 5% for CD56+/PDGFRα+ and CD56+/PDGFRα− cells.

251

Discussion

253   The current study further documents the diversity of aSC in adipose tissue and skeletal muscle of pigs. It also

254   clearly indicates that the diet-induced changes in body composition are associated with changes in the relative

255   proportions of several aSC populations in adipose tissue and skeletal muscle. .

256   Diversity of aSC in adipose tissue and muscle

257   In agreement with our previous work [16], this study shows that cells expressing either CD90, a well-known

258   mesenchymal marker, CD34, an hematopoietic stem cell marker, or CD56, a myogenic marker, were represented

259   at significant levels in both adipose and muscle tissues. It further demonstrates the presence of cells expressing

260   the PDGFRα marker at significant levels in both tissues. PDGFRα is considered as a useful marker to

261   differentiate adipogenic from non-adipogenic cells among interstitial cells with myogenic potential (PW1+

262   interstitial cells) in mice and in human muscle [25, 26]. PDGFRα is also known to be a marker of mesenchymal

263   progenitors distinct from myogenic cells [30, 27]. Among muscle-resident cells, a population of PDGFRα+

264   mesenchymal cells has been identified as progenitors of fibroblasts and adipocytes (fibro/adipogenic progenitors,

265   FAPs) [17,18]. In the current study, cells positive for PDGFRα were more frequent in the perimysium than in

266   the endomysium of muscle, an observation that fitted data reported in humans and mice [17,18]. As reported in

267   white adipose tissue of adult mice [19] PDGFRα+ mesenchymal progenitors were also detected in the stroma of

268   subcutaneous adipose tissue. Indeed, Lee et al. (2012) [20] demonstrated that PDGFRα+ progenitors contributed

269   to white adipogenesis in adipose tissue of mice [20,21]. We further show that the proportions of cell populations

270   expressing PDGFRα and CD56 or PDGFRα and CD90 markers were significant in both tissues as already

271   reported in human muscle [18] and adipose tissue [21] with high expression of CD90+ and PDGFRα+ cells in

272   adipose tissue and high expression of CD90+ and CD56+ cells in skeletal muscle. Importantly, we showed that

273   CD56-/PDGFRα+ cells were mainly adipogenic whatever they originated from adipose tissue and muscle,

274 whereas they had a capacity for myogenic differentiation only when harvested from muscle. Similarly,

275 CD56+/PDGFRα+ and CD56+/PDGFRα− cells also exhibited a tissue-specific differentiation potential. In

276 agreement with our data, it has been shown that (PDGFRα−/CD56+ cells isolated from human muscle biopsies

277 only differentiated into myotubes whereas PDGFRα+/CD56− cells differentiated into adipocytes or fibroblast-

278 like cells [22]. Moreover, both CD56+/PDGFR- and CD56+/PDGFR+ cells exhibited a high myogenic potential

279 in skeletal muscle of adult human donors [23]. Taken together, we suggest that PDGFRα could be a relevant

280 marker to sort cells with predominant adipogenic properties in adipose tissue, as it has been already

281 demonstrated [24], but cannot be a relevant marker to differentiate adipogenic from myogenic cells in skeletal

282 muscle. Moreover, CD56, a marker of myogenic commitment in skeletal muscle [16,25], appeared as the best

283 marker to discriminate mesenchymal markers from myogenic cells in pig skeletal muscle. However, CD56

284 cannot be considered as a marker of myogenic commitment in adipose tissue. Indeed, cells positive for

285 CD56also displayed adipogenic capacities in adipose tissue, in agreement with previous observations [17].

286 Moreover, the presence of CD56+ in adipose cells was not associated with a significant capacity for myogenic

287 differentiation. Other authors have shown that CD56+cells from adipose tissue in adult human were prone to

288 enter in the myogenic program but could not undergo terminal differentiation [32].

289 Changes in aSC due to diet remain scarce and even more in early growth stages.

290 We provided evidence that the reduction of body weight and fatness induced by the HF diet compared with the

291 LF diet [26] was associated with changes in aSC populations in adipose tissue and muscle of growing pigs. This

292 suggests that aSC may be a relevant target to influence later body composition. With the role of programming

293 events in the etiology of obesity, it is important to determine how early nutrition can affect long-term tissue

294 function and systemic metabolism [25,26]. The dietary-induced changes in the proportion of several cell

295 populations are consistent with studies demonstrating that the composition of the aSC compartment of adipose

296 tissue or skeletal muscle is affected in obese adults [27,28,29]. The current study reveals that two aSC

297 populations, CD56+/PDGFRα+ and CD90+/PDGFRα+ cells, were found to be affected by the diet in both

298 adipose tissue and skeletal muscle but in an opposite manner. In adipose tissue, the proportions of these two

299 populations declined with the decrease in backfat percentage induced by the HF diet. In muscle, their proportions

300 increased with the lean mass. The decreased proportion of these cells in adipose tissue may result from a release

301 of these cells as suggested very recently in mice [25]. These types of cells, as well as their activity undergo a

302 modulation related to the variation of body composition during growth. Indeed, there is a strong link between

303 stem cells, preadipocyte differentiation and hyperplasia. This process gives a protection of adipose tissue even in

304   obesity case. The regulation of hyperplasic adipocytes may exert beneficial effects against adipocyte

305   hypertrophy and subsequent insulin resistance [26]. So we can suggest that in our case hyperplasia is regulated at

306   the stem cell level like it has been previously reported in mice [27].

307   In conclusion, the current study clearly shows the link between nutrition, stem cells and the final body

308   composition of animals. It suggests that these cell populations, specifically cells that have opposite regulation in

309   skeletal muscle and adipose tissue (CD90+/PDGFRα+, CD56+/PDGFRα+), can be targeted to modulate body

310   composition.

311

312

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397   **FIGURE LEGENDS**

398   FIGURE 1. Representative immunohistochemical pictures showing in situ localization of stroma vascular cells

399   in subcutaneous adipose tissue (A) and skeletal muscle (B) of pigs. Cross-sections were stained in red for

400   PDGFRα cell surface marker with a blue DAPI counterstaining to localize nuclei. An antibody recognizing

401   laminin (green) was also used to stain the basal membrane. In subcutaneous adipose tissue, white arrows

402   indicated stromal and perivascular structures and grey arrows indicate unstained satellite cells in skeletal muscle.

403   Scale bar 20 μm.

404

405   FIGURE 2. Expression of CD34, CD56, CD90 and PDGFRα markers in stromal vascular cells isolated from

406   subcutaneous adipose tissue (SCAT, A) and skeletal muscle (B) of pigs with low (RFI-) or high residual feed

407   intake (RFI+) fed a high-fat, high-fiber (HF) diet and low-fat, low-fiber (LF)

408   After cell isolation, percentages of positive cells for CD34 Phycoerythrin (PE), CD56 Phycoerythrin-Cyanine

409   7(PE-Cy7), CD90 Allophycocyanin (APC), and PDGFRα Fluorescein isothiocyanate (FITC) were determined

410   by flow cytometry. Percentages of positive cells are expressed as means ± SE (n=5 animals/ group). For each

411   marker, means with different letters differed, $P < 0.05$.

412

413   FIGURE 3. Expression of CD34 Phycoerythrin (PE), CD56 Phycoerythrin-Cyanine 7(PE-Cy7), CD90

414   Allophycocyanin (APC)and PDGFRα Fluorescein isothiocyanate (FITC) markers in stromal vascular cells

415   isolated from subcutaneous adipose tissue (SCAT, A) and skeletal muscle (B) of pigs with low (RFI-) (white

416   bars) or high residual feed intake (RFI+) (black bars) fed a high-fat, high-fiber (HF) diet and low-fat, low-fiber

417   (LF).

418   After cell isolation, percentages of cells expressing phenotypes : CD34+/PDGFRα+, CD90+/PDGFRα+,

419   CD56+/PDGFRα-, CD56+/PDGFRα+, and CD56-/PDGFRα+ of total isolated cells were determined by flow

420   cytometry. Percentages of cell populations are expressed as means ± SE (n=5 animals/ group). For each marker,

421   means with different letters differed, $P < 0.05$.

422

423   FIGURE 4. Representative flow cytometry analyses of cells isolated from subcutaneous adipose tissue (A)

424   before and (B) after cell sorting for CD56-PE Cy. Representative flow cytometry analyses of cells isolated from

425   skeletal muscle tissue before (C) and after (D) cell sorting for CD56-PE Cy. Each representation is illustrated by

426   the isotype control (up) associated to its corresponding positive staining (down).

427

428   FIGURE 5. Cells isolated from adipose tissue and muscle of RFI- pigs fed the LF diet were considered as

429   controls. (A) Typical morphology of stromal vascular (SV) cells originating from (A) subcutaneous adipose

430   tissue (SCAT) and (B) skeletal muscle of RFI- pigs fed the LF diet after nine days of culture. Cells were cultured

431   for 9 days in adipogenic or myogenic media and day 0 corresponded to cell seeding. Adipogenic differentiation

15

was evaluated with Oil Red O staining and myogenic differentiation with desmin and myosin heavy chain (MF20) immunostaining. Cell morphology was further examined using phase contrast on D9 and arrows indicate lipid droplets. Scale bar 10 μm.

Table 1 Ingredients, chemical and nutritional compositions of the experimental diets[1]

	Growing		Finishing	
Items	HF	LF	HF	LF
Ingredients, g/kg (as fed basis)				

Wheat	269.5	362.2	330.9	417.2
Barley	269.5	362.2	330.9	417.2
Soybean meal	233.5	199.9	116.1	84.9
Corn starch	0	27.5	0	35.7
Crushed wheat straw	114.5	0	112.6	0
Rapeseed oil	25.1	3.4	25.1	3.5
Soybean oil	50.1	6.8	50.3	6.7
L-Lysine HCl	2.8	3.2	2.9	3.3
L-Threonine	1.17	1.20	10.3	10.5
L-Tryptophane	0.28	0.26	0.22	0.19
DL-Methionine	1.04	0.76	0.46	0.38
Salt	4.5	4.50	4.50	4.50
Calcium carbonate	10.5	10.5	10.0	10.0
Dicalcium phosphate	12.5	12.5	10.0	10.0
Vitamins and minerals premix	5.0	5.0	5.0	5.0
DM, g/100 g	88.0	87.4	87.2	86.9
Chemical composition, % (as fed basis)				
Ash	5.4	5.0	4.7	4.3

OM	82.6	82.4	82.5	82.6
CP	17.3	17.4	13.1	13.4
Fat	7.0	2.1	7.4	2.2
Starch	30.8	42.6	36.2	48.5
NDF	17.7	12.8	18.3	11.5
ADF	8.5	3.8	7.9	3.5
GE, MJ/kg	17.5	15.9	17.3	15.8
Nutritional values				
ME, MJ/kg	12.9	12.9	12.9	12.9
NE, MJ/kg	9.8	9.6	10.0	9.9
Digestible lysine, g/kg	9.5	9.5	7.0	7.0
Digestible threonine, g/kg	0.62	0.62	0.46	0.46
Digestible methionine, g/kg	0.32	0.30	0.21	0.21
Fatty acid (FA) composition, % extracted FA				
C18:2 n-6	41.0	45.3	41.6	44.6
C18:3 n-3	5.6	5.0	5.7	4.7
Saturated FA	13.9	18.5	13.8	19.0
Mono-unsaturated FA	36.2	27.0	36.4	25.9

Poly-unsaturated FA	54.6	49.8	50.0	55.1
Ratio n-6: n-3	5.0	5.3	5.4	4.8

448 [1]A low fat low fiber diet (LF) and a high fat high fiber diet (HF) were formulated at the same

449 protein and metabolizable energy (ME) contents. For each dietary group, a growing diet was

450 distributed to pigs from 76 d of age onwards for 6 weeks, and a finishing diet which differed

451 for the protein and amino-acid contents, was distributed thereafter and until slaughter.

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462 **Table 2** List of antibodies used for flow cytometry (FACS) analysis or immunohistochemistry

Marker	Isotype	Fluorochrome[1]	Clone	Supplier
CD11b	mouse IgG1	PE-Cy7	ICRF44	Biolegend
CD14	mouse IgG2a	RPE-alexa647	TUK4	AbD sérotec
CD31	mouse IgG1	RPE	LCI-4	AbD sérotec
CD34	mouse IgG1	PE	563	BD Biosciences
CD45	mouse IgG1	FITC	K252-1E4	AbD sérotec
CD56	mouse IgG2b	PE-Cy7	NCAM 16.2	BD Biosciences
CD56	mouse IgG2b	APC	NCAM 16.2	BD Biosciences
CD90	mouse IgG1	APC	5E10	BD Biosciences
PDGFRα	Goat IgG	Unconjugated	Polyclonal	Santa Cruz
Laminin	Rabbit	Unconjugated	Polyclonal	Sigma

[1] Fluorochromes for FACS analysis, APC, Allophycocyanine, BD, Becton Dickinson; CD, Cluster of Differentiation; Cy7, Cyanine 7; FITC, fluorescein isothiocyanate; IgG, Immunoglobulin G; PE, PhycoErythrin; RPE, R-PhycoErythrin.

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482 **Table 3** Performance of pigs with low (RFI-) or high residual feed intake (RFI+) fed a low-fat,

483 high-fat, high-fiber (HF) or low-fiber (LF) diet

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Diet	HF		LF			Effects	
Genotype	RFI-	RFI+	RFI-	RFI+	RSD	Diet	Line
n	6	6	6	6	-	-	-
Final live weight, kg	70.6	69.7	81.7	69.2	4.2	<0.001	0.007
ADG, g/d	826.6	739.5	990.9	856.4	71	<0.001	<0.001
ADFI, g/d	2134	2121	2550	2373	602	0.01	0.01
Composition,% of chilled carcass weight							
Backfat	5.7	6.2	8.0	8.5	0.85	<0.001	0.51
Loin	28.7	28.3	28.3	27.6	0.41	<0.05	0.07
Loin/Backfat	4.83	4.85	3.88	3.42	0.8	<0.001	0.57

485

486 [1]Value are least squares means for the effects of line and diet. There was no interaction between RFI

487 line and diet for any analyzed traits. RSD, Relative Standard Deviation.

488 [2]There was no interaction ($P > 0.10$) between genetic line (high or low residual feed intake) and diet

489 (HF: high fiber high fat; LF: low fiber low fat) for any analyzed traits. Lsmeans for the effects of Line

490 and of Diet are shown. Average daily gain (ADG), average daily feed intake (ADFI) of growing pigs.

491 **Table 4** Correlations between the percentage of cell populations in subcutaneous adipose tissue

492 (SCAT) relative to the backfat weight, and between the cell populations in skeletal muscle and the loin

493 weight.

Cell populations	Backfat weight		Loin weight	
	r^a	*P* value	r^a	*P* value
CD34+/PDGFRα-	-0.37	0.10	0.69	<0.001
CD34- /PDGFRα+	0.53	<0.05	-0.68	<0.05
CD56+ /PDGFRα+	0.59	<0.05	-0.55	<0.05
CD90+ /PDGFRα+	0.62	<0.05	-0.57	<0.05

494 r^a square root of R^2 obtained for linear regression analysis

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506 **Suppl Table 1** Percentage of cell populations in subcutaneous adipose tissue (SCAT) and skeletal

507 muscle of pigs with low (RFI-) or high residual feed intake (RFI+) fed a low-fat, low-fiber

508 (LF) or high-fat, high-fiber (HF) diet.

	HF		LF		RSD	Diet	Line	Diet * Line
	RFI-	RFI+	RFI-	RFI+				
SCAT								
CD34+/PDGFRα-	0.33	0.60	0.10	0.19	0.08	0.09	0.47	0.17
CD34+/PDGFRα+	0.86	0.80	0.65	0.83	0.09	0.18	0.41	0.28
CD34-/PDGFRα+	21.8	29.4	32.8	31.1	4.61	<0.001	<0.001	<0.001
CD56+/PDGFRα-	0.5	0.6	0.4	0.4	0.11	0.33	0.22	0.86
CD56+/PDGFRα+	7.0	8.9	14.4	12.6	6.73	<0.001	0.96	0.13
CD56-/PDGFRα+	15.6	21.4	17.8	19.4	13.94	0.95	<0.05	0.23
CD90+/PDGFRα-	16.1	8.3	6.1	4.3	21.86	<0.05	<0.05	0.18
CD90+/PDGFRα+	19.7	21.2	23.8	26.8	16.97	<0.05	0.24	0.70
CD90-/PDGFRα+	2.9	9.0	8.4	5.2	14.49	0.64	0.42	<0.05
Muscle								
CD34+/PDGFRα-	4.0	1.4	11.0	2.7	4.92	<0.05	<0.05	0.05
CD34+/PDGFRα+	8.4	5.0	6.7	5.5	2.37	0.34	<0.05	0.11
CD34-/PDGFRα+	4.6	5.6	1.7	1.0	2.31	<0.05	0.37	0.61
CD56+/PDGFRα-	12	15.09	18.9	17.9	12.33	<0.05	0.54	0.22
CD56+/PDGFRα+	11.2	8.4	6.4	6.4	2.36	<0.001	0.06	0.57
CD56-/PDGFRα+	1.8	2.2	1.7	1.0	0.39	<0.05	0.67	0.06
CD90+/PDGFRα-	9.6	20.0	8.4	21.3	13.94	0.26	<0.001	0.70
CD90+/PDGFRα+	11.3	9.9	7.5	6.9	2.05	<0.001	0.15	0.57
CD90-/PDGFRα+	0.48	1.70	0.6	0.5	0.7	<0.05	0.11	0.17

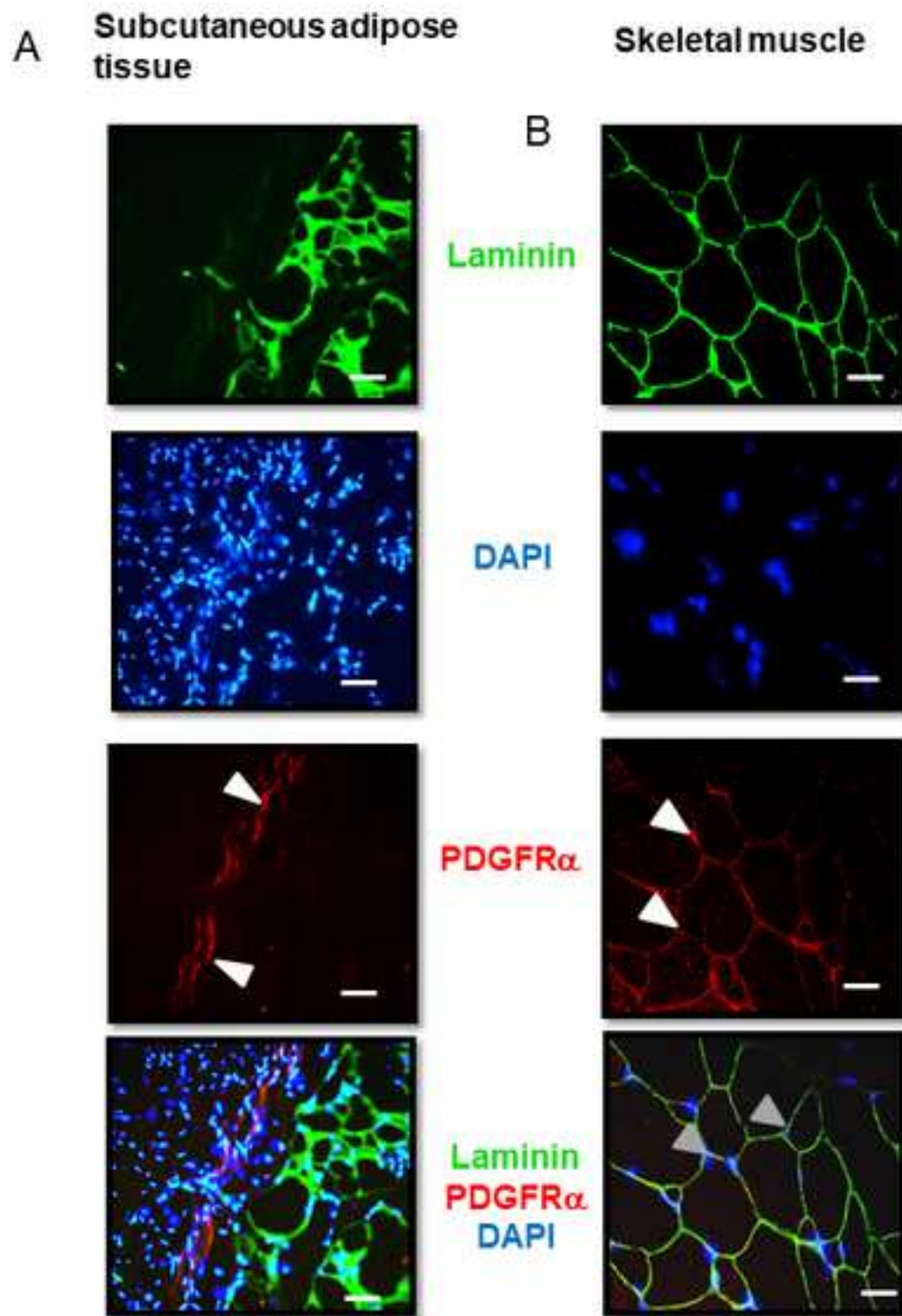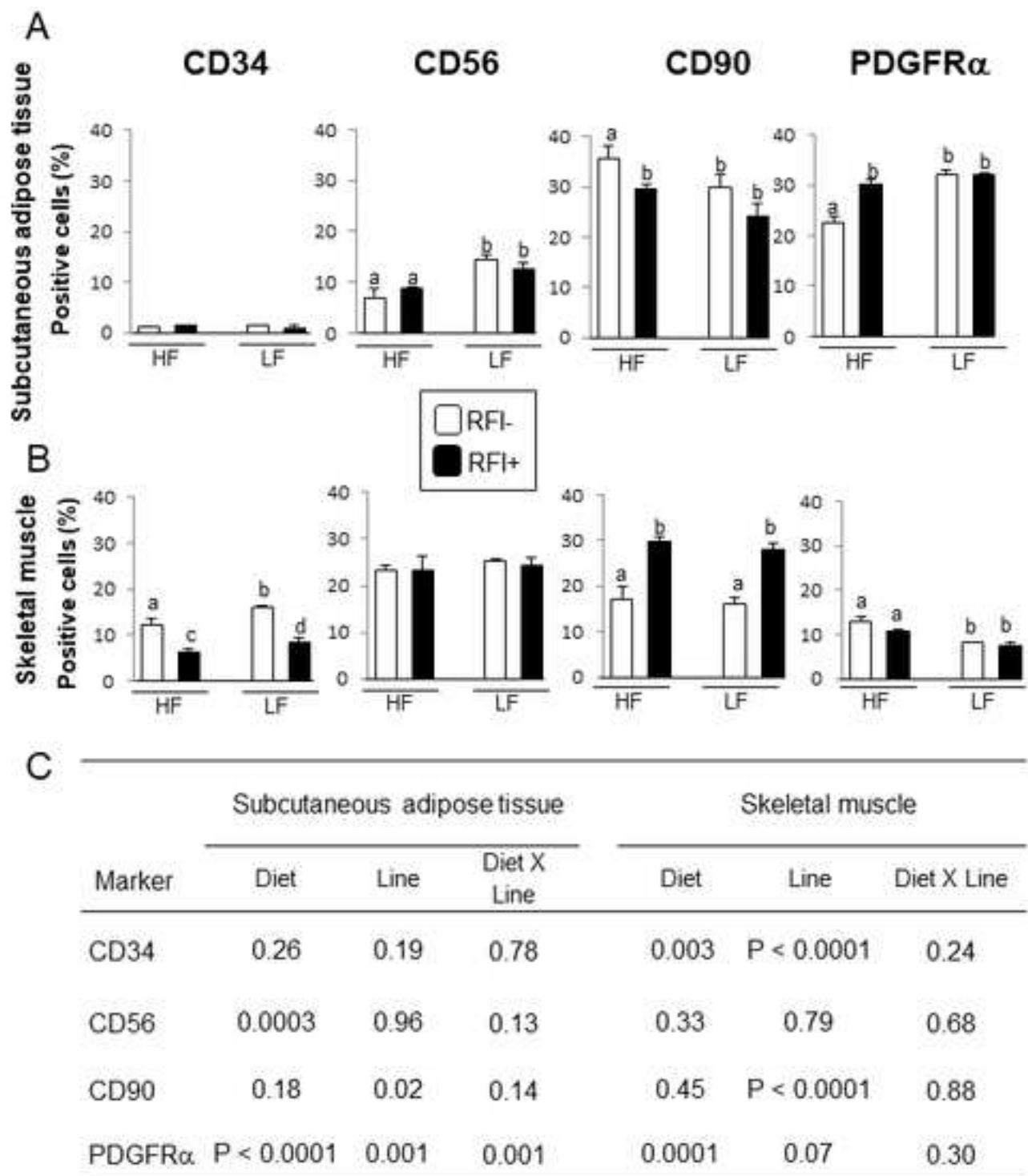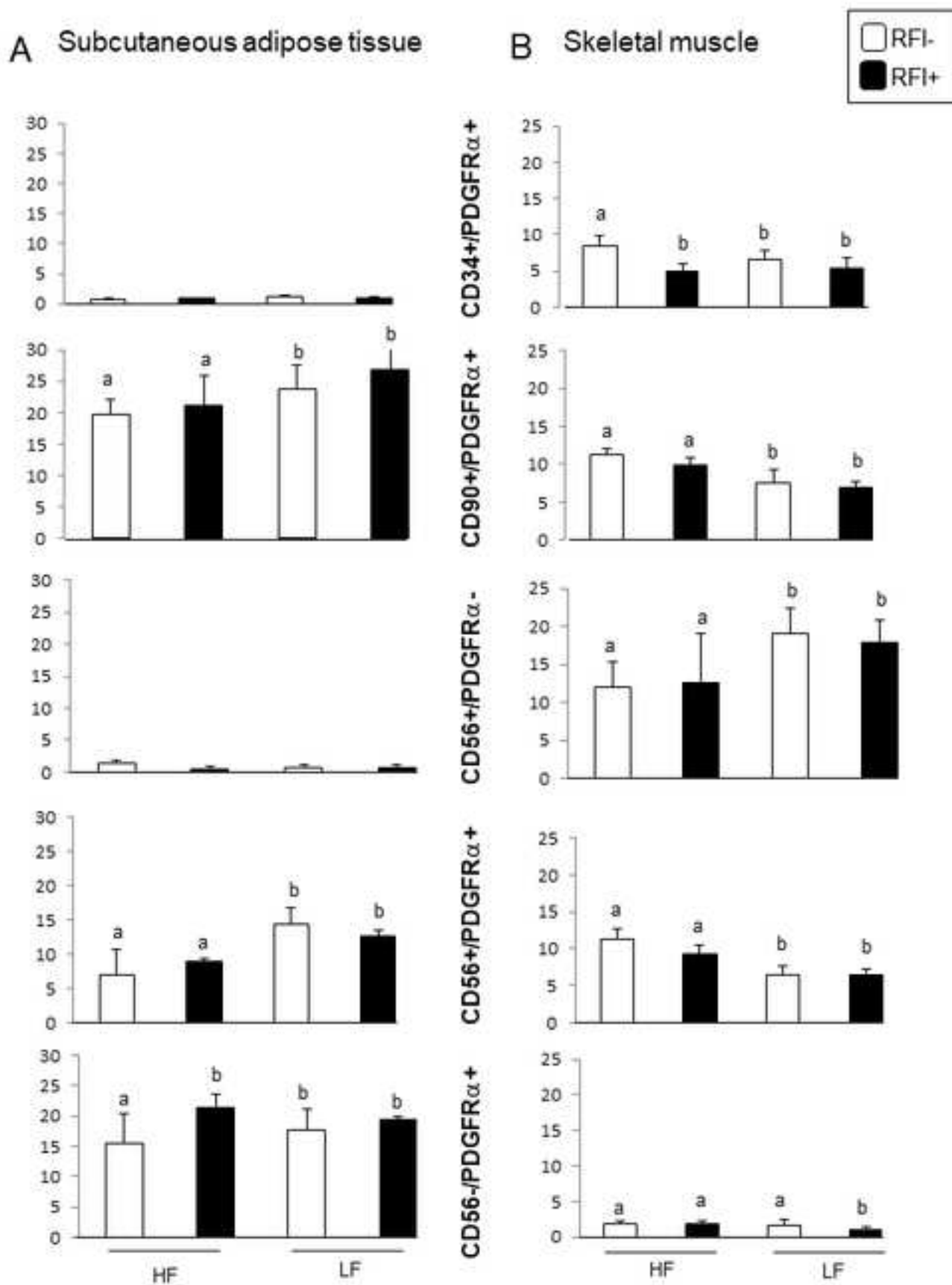509 [1]Values are means expressed as a percentage of cell phenotyping (n=6 animals/age group).

Figure 1 Click here to access/download;Figure;Fig1.TIF

A Subcutaneous adipose tissue

B Skeletal muscle

Laminin

DAPI

PDGFRα

Laminin
PDGFRα
DAPI

FIGURE 1

Figure 2

Click here to access/download;Figure;Fig2.TIF ±

A Subcutaneous adipose tissue — Positive cells (%): CD34, CD56, CD90, PDGFRα (HF, LF; RFI−, RFI+)

B Skeletal muscle — Positive cells (%): CD34, CD56, CD90, PDGFRα (HF, LF; RFI−, RFI+)

Legend: □ RFI− ■ RFI+

C

Marker	Subcutaneous adipose tissue			Skeletal muscle		
	Diet	Line	Diet X Line	Diet	Line	Diet X Line
CD34	0.26	0.19	0.78	0.003	P < 0.0001	0.24
CD56	0.0003	0.96	0.13	0.33	0.79	0.68
CD90	0.18	0.02	0.14	0.45	P < 0.0001	0.88
PDGFRα	P < 0.0001	0.001	0.001	0.0001	0.07	0.30

FIGURE 2

FIGURE 3

Figure 4

Click here to access/download;Figure;Fig4.TIF ±

FIGURE 4

Figure 5

FIGURE 5