Hygiene of housing conditions and proinflammatory signals alter gene expressions in porcine adipose tissues and blood cells

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Adipose tissue is an organ with metabolic, endocrine and immune functions. In this tissue, the expressions of genes associated with several metabolic pathways, including lipid metabolism, have been shown to be affected by genetic selection for feed efficiency, an important trait to consider in livestock. We hypothesized that the stimulation of immune system caused by poor hygiene conditions of housing impacts the molecular and cellular features of adipose tissue and that the impact may differ between pigs that diverge in feed efficiency. At the age of 12 weeks, Large White pigs from two genetic lines divergent for residual feed intake (RFI) were housed in two contrasting hygiene conditions (good vs poor). After six weeks of exposure, pigs were slaughtered (n = 36). Samples of blood, subcutaneous (SCAT) and perirenal (PRAT) adipose tissues were collected for cell response and gene expression investigations. The decrease in the relative weight of PRAT was associated with a decline in mRNA levels of *FASN*, *ME*, *LCN2* and *TLR4* (P < 0.05) in pigs housed in poor conditions compared with pigs housed in good conditions for both RFI lines. In SCAT, the expressions of only two key genes (*PPARG* and *TLR4*) were significantly affected by the hygiene of housing conditions. Besides, the mRNA levels of both *LCN2* and *GPX3* were influenced by the RFI line (P < 0.05). Because we suspected an effect of poor hygiene at the cellular levels, we investigated the differentiation of stromal vascular cells isolated from SCAT *in vitro* in the absence or presence of a pro-inflammatory cytokine, Tumor Necrosis Factor-α (TNF-α). The ability of these cells to differentiate in the absence or presence of TNF-α did not differ among the four groups of animals (P > 0.05). We also investigated the expressions of genes involved in the immune response and lipid metabolism in whole blood cells cultured in the absence and presence of LPS. The hygiene conditions had no effect but, the relative expression of the *GPX3* gene was higher (P < 0.001) in high RFI than in low RFI pigs while the expressions of *IL-10* (P = 0.027), *TGFβ1* (P = 0.023) and *ADIPOR2* (P = 0.05) genes were lower in high RFI than in low RFI pigs.

Overall, the current study indicates that the hygiene of housing had similar effects on both RFI lines on the expression of genes in adipose tissues and on the features of SCAT adipose cells and whole blood cells in response to TNF-α and LPS. It further demonstrates that the number of genes with expression impacted by housing conditions was higher in PRAT than in SCAT. It suggests a depot-specific response of adipose tissue to the current challenge.

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7 Short title: Housing and pig adipose tissue

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Abstract

Adipose tissue is an organ with metabolic, endocrine and immune functions. In this tissue, the expressions of genes associated with several metabolic pathways, including lipid metabolism, have been shown to be affected by genetic selection for feed efficiency, an important trait to consider in livestock. We hypothesized that the stimulation of immune system caused by poor hygiene conditions of housing impacts the molecular and cellular features of adipose tissue and that the impact may differ between pigs that diverge in feed efficiency. At the age of 12 weeks, Large White pigs from two genetic lines divergent for residual feed intake (RFI) were housed in two contrasting hygiene conditions (good vs poor). After six weeks of exposure, pigs were slaughtered (n = 36). Samples of blood, subcutaneous (SCAT) and perirenal (PRAT) adipose tissues were collected for cell response and gene expression investigations. The decrease in the relative weight of PRAT was associated with a decline in mRNA levels of *FASN*, *ME*, *LCN2* and *TLR4* (P < 0.05) in pigs housed in poor conditions compared with pigs housed in good conditions for both RFI lines. In SCAT, the expressions of only two key genes (*PPARG* and *TLR4*) were significantly affected by the hygiene of housing conditions. Besides, the mRNA levels of both *LCN2* and *GPX3* were influenced by the RFI line (P < 0.05). Because we suspected an effect of poor hygiene at the cellular levels, we investigated the differentiation of stromal vascular cells isolated from SCAT *in vitro* in the absence or presence of a pro-inflammatory cytokine, Tumor Necrosis Factor-α (TNF-α). The ability of these cells to differentiate in the absence or presence of TNF-α did not differ among the four groups of animals (P > 0.05). We also investigated the expressions of genes involved in the immune response and lipid metabolism in whole blood cells cultured in the absence and presence of LPS. The hygiene conditions had no effect but, the relative expression of the *GPX3* gene was higher (P < 0.001) in high RFI than in low RFI pigs while the expressions of *IL-10* (P = 0.027), *TGFβ1* (P = 0.023) and *ADIPOR2* (P = 0.05) genes were lower in high RFI than in low RFI pigs. Overall, the current study indicates that the hygiene of housing had similar effects on both RFI lines on the expression of genes in adipose tissues and on the features of SCAT adipose cells and whole blood cells in response to TNF-α and LPS. It further demonstrates that the number of genes with expression impacted by housing conditions was higher in PRAT than in SCAT. It suggests a depot-specific response of adipose tissue to the current challenge.

Introduction

Farm animals like pigs are exposed to multiple environmental stimuli during their growth (Colditz & Hine, 2016), some of them have been clearly identified to be risk factors of poor health. For example, poor hygiene of housing results in degraded health, reduced growth performance, and immune system activation, characterized by a low-grade inflammation (Chatelet et al., 2018). This inflammatory response relies on the production of proinflammatory cytokines such as interleukin-6 (IL-6) or Tumor Necrosis factor α (TNF-α), which contribute to the redirection of nutrients to the immune system at the expense of muscle and adipose tissues (Gabler & Spurlock, 2008; Ajuwon, 2014).

Initially, white adipose tissue was considered as a preferential site of energy storage in the form of triacylglycerols (Ameer et al., 2014). Now, it is also recognized as an endocrine organ that produces a large number of secretory products termed adipokines or adipocytokines, either from adipocytes or from cells of the stromal vascular fraction, which contains preadipocytes, lymphocytes, macrophages, fibroblasts and vascular cells (Louveau et al., 2016). These adipokines, such as leptin, adiponectin, insulin-like growth factor-I (IGF-I) but also IL-6, IL-10 or TNF-α, communicate with other organs including brain, liver, skeletal muscle, the immune system, and adipose tissue itself (Fantuzzi, 2005; Ramsay & Caperna, 2009). Their expression can depend on the anatomical location (subcutaneous or visceral) of adipose tissues (Tchkonia et al., 2013). In addition, an increase in body fat mass may trigger the development of an inflammatory state (Stolarczyk, 2017). Therefore, the biological processes underlying the contribution of adipose tissue to an inflammatory response and hence the ability of pigs to sustain sanitary challenges deserves further studies (Patience, Rossoni-Serão & Gutiérrez, 2015).

Selection of pigs for residual feed intake (RFI) has been used to improve feed efficiency. Briefly, for a similar production level, high RFI (HRFI) pigs eat more than predicted based on average requirements for growth and maintenance and therefore are less efficient than low RFI (LRFI) pigs (Gilbert et al., 2007, 2017). Genetic selection for RFI results in changes in the partition of nutrients between maintenance and growth (Labussière et al., 2015; Merlot, Gilbert & Le Floc'h, 2016). With respect to adipose tissue, this selection induces changes in the expression of genes involved in several pathways including pathways related to lipid metabolism and defense

83 response (Gondret et al., 2017; Horodyska et al., 2019b). These differences in nutrient

84 partitioning may alter pig ability to allocate nutrients for stress and immune responses when

85 facing environmental challenges (Rauw et al., 1998). For example, it was reported that LRFI

86 growing pigs coped better with an immune challenge caused by poor hygiene of the housing than

87 HRFI pigs (Chatelet et al., 2018). We hypothesized that this difference between the two RFI

88 lines may involve changes in the molecular and/or cellular features of adipose tissues and blood

89 immune cells. Therefore, the present study was carried out to evaluate the effects of poor

90 hygiene of housing conditions on the expression of genes in adipose tissue of LRFI and HRFI

91 pigs. *In vitro*, we reproduced inflammatory conditions using an exposure to TNF-α or

92 lipopolysaccharide (LPS), the main structural component of the outer membrane of most Gram-

93 negative bacteria, to determine the impact of inflammatory stimuli and genetic selection on

94 adipocyte differentiation and on the expression of genes involved in immune-adipose

95 communication in blood immune cells.

96

Materials & Methods

97

98 Ethical approval/declaration

99 The experiment was performed in the INRAE UE3P experimental facility (Saint-Gilles, France;

100 (doi.org/10.15454/1.5573932732039927E12) in compliance with the ethical standards of the

101 European Community (Directive 2010/63/EU) and was approved by the regional ethical

102 committee (Comité Rennais d'Ethique en matière d'expérimentation animale or CREEA

103 Rennes). The experiment approval number is APAFIS#494–2015082717314985.

104

105 Animals and sample collection

106 For this experiment, a subset of growing Large White pigs (males and females) was selected

107 from a larger study previously described by Chatelet et al. (2018). Pigs originated from the 8th

108 generation of two lines divergently selected for residual feed intake (RFI), a measure of feed

109 efficiency, were used (Gilbert et al., 2007). They were weaned at 4 weeks of age. At 12 weeks of

110 age, same-sex pairs of pigs were selected within a litter from the high RFI (HRFI, n=16) and the

111 low RFI (LRFI, n=20) lines. Within a pair, animals were then randomly assigned to a room with

112 good hygiene conditions (n= 8 and 10, respectively) and the other half to a room with poor

113   hygiene conditions (n=8 and 10, respectively). Good housing conditions included room cleaning,

114   disinfection, and adoption of strict biosecurity precautions. In contrast, poor hygiene conditions

115   consisted of no cleaning nor sanitation of the room after the previous occupation by non-

116   experimental pigs and during the experiment as fully described previously by Chatelet et al

117   (2018). This model is known to induce the stimulation of body defenses (inflammation, immune

118   response, oxidative status) that is not caused by a specific and single biotic (virus, bacteria,

119   fungi…) or abiotic (gas, dust…) agents. All pigs were housed in individual pens (85 x 265 cm)

120   on concrete floor and have free access to a standard diet formulated to meet or exceed the

121   nutritional requirement of growing pigs (9.47 MJ of net energy/kg, starch 44.2%; fat 3.1%; crude

122   protein 15.3%), and to water. After six weeks in these housing conditions and after an overnight

123   fast, animals were euthanized by electrical stunning and exsanguination. Immediately after

124   slaughter, blood samples were collected from the jugular vein on heparin. Samples of

125   subcutaneous dorsal adipose tissue (SCAT) and perirenal adipose tissue (PRAT) were collected,

126   cut into small pieces, frozen in liquid nitrogen, and stored at -75°C until total RNA extraction.

127   For cell isolation from SCAT, portions of tissues were placed in warm Krebs-Ringer-

128   bicarbonate-HEPES buffer and processed within 30 min (Perruchot et al., 2013).

129

130   Culture of whole blood cells

131   Whole blood cells were incubated in resting conditions (medium alone) and after activation with

132   LPS (O55:B5, Sigma-Aldrich, France). This molecule is present on the wall of Gram-negative

133   bacteria and induces the production of inflammatory mediators by immune cells via the activation

134   of Toll-like receptor 4 (TLR4). Whole heparinized blood samples were diluted 1:5 in complete

135   cell culture medium (RPMI supplemented with 10% fetal bovine serum (FBS), 1% L-Glutamine,

136   and 1% penicillin/streptomycin), and distributed in 24-well culture plates (0.4 mL/well) in

137   triplicates. Complete medium (0.6 mL) alone or supplemented with LPS (Sigma, 10 µg/mL in the

138   well) was then added. After 20 h of incubation in 95% air-5 % CO_2 at 37°C, cells were collected,

139   triplicates were pooled, spun down, suspended in 0.5 mL of lysis buffer DL (Macherey-Nagel,

140   France) and stored at -75°C.

141

142   Adipose cell isolation

143   Cells were isolated from fresh subcutaneous dorsal adipose tissue by collagenase digestion as

144   previously described (De clercq et al., 1997; Perruchot et al., 2013). Briefly, fresh adipose tissue

145   was cut into small pieces and dissociated by enzymatic digestion with collagenase II and XI

146   (Sigma, France) under shaking in a dry bath for 45 min at 37°C. Then, a centrifugation at 400 g

147   for 10 min was performed to separate floating adipocytes from the pellet of stromal vascular

148   fraction (SVF) cells. After resuspension, SVF cells were successively filtered through 200-μm

149   and 25-μm nylon membranes (Dutscher, France). After isolation, SVF cells were placed in

150   FBS/10% dimethyl sulfoxide (DMSO, Sigma) and frozen at -150°C.

151

152   Culture of stromal vascular fraction cells and differentiation induction

153   First, individual vials of $1x10^6$ cryopreserved SVF cells were thawed in a 37°C water bath (2–3

154   min). Then, cells were grown at 37°C under 95% air-5% CO_2 and were first placed in

155   Dulbecco's modified Eagle's medium (DMEM 1X, GlutaMAX; with 4,5 g/L glucose, Gibco,

156   Invitrogen, USA) supplemented with 20% FBS and 50 U/mL Penicillin-Streptomycin (Gibco,

157   Invitrogen) until confluence in T25 cell culture flask (Nunc EasYFlask 25 cm^2, Thermo Fisher

158   Scientific, France). Then, at day 0 (D0), cells were seeded in six-well plates (NUNCLON™

159   Delta Surface, Thermo Fisher Scientific), at a density of 2.8 x 10^5 cells/well (9.6 cm^2/well),

160   coated with growth factor reduced Matrigel (1/50 vol/vol, Corning, Boulogne-Billancourt,

161   France) as described previously (Perruchot et al., 2013). From D4 to D6 of culture, the medium

162   was changed and replaced by DMEM, GlutaMAX, high glucose (Gibco, Invitrogen)

163   supplemented with 2.5% swine serum (Gibco, Invitrogen), 50 U/mL Penicillin-Streptomycin

164   (Gibco, Invitrogen), 2.6 nM porcine insulin (Sigma, Lyon, France) and 100 nM Cortisol (Sigma).

165   On D6, cells were placed in a medium allowing adipocyte differentiation. This culture medium

166   consisted of DMEM, GlutaMAX, high glucose supplemented with 50 U/mL Penicillin-

167   Streptomycin, 100 nM 3-Isobutyl-1-methylanxthine (IBMX; Sigma), 2.6 nM insulin, 10 μM

168   dexamethasone (Sigma), 0.2 nM T3 (Sigma), 10 μg/mL transferrin (Sigma), 100 μM ascorbic

169   acid (Sigma) and 100 nM Cortisol (Sigma). The differentiation culture medium was renewed

170   every two to three days. From D4 to D22 of culture, cells seeded to study differentiation were

171   treated without (control condition) or with 1 ng/mL or 10 ng/mL of recombinant porcine TNF-α

172   (Invitrogen).

173   In order to assess the percentage of cell differentiation, pictures were taken on D14, D18 and/or

174   D22 of culture using a phase contrast microscope at X40 magnification (AxioVert A1, Zeiss,

175   Oberkochen, Germany). Lipid accumulation in cells was estimated from two wells per condition

176   tested for each animal and three random fields per well with ImageJ software (ImageJ 1.50i;

177   National Institutes of Health, Bethesda, MD, USA). Results were expressed as surface ratio of

178   lipid droplets present in the cells divided by the total area of cells in the same field.

179

180   Total RNA extraction from whole blood cells and adipose tissues

181   For whole blood cells, total RNAs were extracted using a commercial kit (NucleoSpin RNA

182   blood kit, Macherey-Nagel) according to the manufacturer's instructions. After a DNase

183   treatment (DNA-free kit, Applied Biosystems, Foster City, CA, USA) in the presence of a RNase

184   inhibitor (ThermoFisher Scientific), the quality and amount of extracted RNA were estimated

185   using a microvolume DS-11 spectrophotometer (DeNovix, Clinisciences, France). After a

186   concentration step performed with a speed-vac concentrator (ThermoFisher Scientific), only

187   samples reaching the minimal required RNA concentration of 111 ng/µL were used. For both

188   adipose tissues, total RNAs were extracted using RNeasy Lipid Tissue Mini Kit (Qiagen, France)

189   according to the manufacturer's instructions, and were quantified using the DS-11

190   spectrophotometer. The integrity of extracted total RNA from whole blood cells and adipose

191   tissues was assessed using the RNA 6000 Nano kit (Agilent Technologies France) with the

192   Agilent 2100 Bioanalyzer (Agilent Technologies) and samples meeting quality criteria were kept

193   for further analyses. Ratios of A260/280 and A260/230 were greater than 1.6. RNA integrity

194   numbers were between 7.0 and 10.0.

195

196   Quantitative real-time PCR (qPCR)

197   For the determination of the expression levels of genes in blood cells and in adipose tissues, first-

198   strand cDNA synthesis was performed with 1 µg of total RNA, by using High Capacity RNA to

199   cDNA kit according to the manufacturer's instructions (Applied Biosystems, Foster City, CA,

200   USA). Primers (Table 1) were designed from porcine sequences available in Ensembl or NCBI

201   databases using Primer Express® v3.0 software (Applied Biosystems).

202   For blood cell samples, amplification reactions and disassociation curves were carried out on a

203   Step One Plus TM real-time PCR system (Applied Biosystems). Among the seven tested house-

204   keeping genes (*B2M, GAPDH, HRPT1, PPIA, TBP1, YWHAZ, 18S*), *B2M, PPIA and TBP1* were

205   used to calculate the normalization factor. They were identified as the most stable house-keeping

206   genes by the RefFinder algorithm (https://github.com/SEAL-UCLA/Ref-Finder) and their

207   expression cycle threshold was not influenced by the effects of line and housing conditions.

208

209   Expression levels of genes in adipose tissues were determined using the SmartChip Real-Time

210   PCR system with a 5184-well chip (TaKaRa, Saint-Germain-en-Laye, France) available at the

211   EcogenO Genomic Platform (https://ecogeno.univ-rennes1.fr/ ; OSUR, Rennes, France).

212   Amplification reactions were carried out using LightCycler 480 SYBR Green 1 Master (Roche

213   Diagnostics, France) with a final cDNA concentration of 1 ng/µL and a primer concentration of

214   500 nM dispensed using the SmartChip Multisample Nanodispenser. Amplification conditions

215   were as follows: 5 min at 95°C followed by 45 cycles of 30 sec at 95°C, 30 s at 60°C and 30 sec

216   at 72°C, followed by 15 sec at 95°C and 1 min at 60°C. Specificity of the amplification products

217   was checked by dissociation curve analysis. *YWHAZ* and *PPIA* were identified as the most stable

218   housekeeping genes and were used for normalization. For all the examined genes, the normalized

219   expression level N was calculated according to the following formula: $N = E^{-\Delta Cq(sample\text{-}calibrator)}/NF$

220   where E is calculated from the slope of calibration curve, Cq is the quantification

221   cycle, calibrator is a pool of all samples and NF is the normalized factor calculated using the

222   geNorm algorithm. For all studied genes, E was between 1.68 and 2.10.

223

224   Statistical analyzes

225   Data were subjected to analysis of variance (ANOVA) considering the individual pig as the

226   experimental unit. For data related to adipose tissues, statistical analyzes were performed using

227   the GraphPad Prism software (version 8.4.3 (686), San Diego, CA, USA). For data related to

228   gene expressions, hygiene conditions, RFI line and their interactions were considered as fixed

229   effects. For data related to the response of adipose cells to TNF-α, hygiene conditions, RFI line,

230   TNF-α dose and their interactions were considered as fixed effects. For whole blood cells,

231   hygiene conditions, RFI line, presence of LPS and their interactions were considered as fixed

232   effects, the pig was used as a random factor, and ANOVA was performed using R software

233   (version 3.5.1). Results are presented as mean ± standard error of mean (SEM). Differences were

234   considered statistically significant if P ≤ 0.05 and were discussed as a trend if 0.05 < P < 0.1.

235

Results

237   Growth performance and body composition

238   Growth performance and body composition of selected pigs have been previously described in

239   Sierzant et al. (2019). Briefly, average daily gain was significantly lower in HRFI pigs housed in

240   poor hygiene conditions than in HRFI pigs housed in good hygiene conditions with no

241   significant difference between LRFI pigs. The relative weight of PRAT was significantly lower

242   in poor hygiene pigs than in good hygiene pigs for both RFI lines. The proportion of SCAT in

243   both RFI lines was not influenced by hygiene of housing conditions.

244

245   Expression of genes in adipose tissues

246   The relative expressions of genes related to adipocyte differentiation, metabolism, secretory

247   functions, oxidative stress and pathogen recognition, were determined in perirenal (PRAT) and

248   subcutaneous (SCAT) adipose tissues. Data related to genes with relative expressions

249   significantly affected by housing conditions and/or RFI selection in PRAT and/or in SCAT are

250   shown in Tables 2 and 3. Other data are presented in supplementary Tables 1 and 2. First, there

251   was no significant interaction between housing conditions and RFI lines (Tables 2 and 3) with

252   the exception of the *COX1* gene in SCAT.

253   In PRAT, the relative expressions of genes involved in lipogenesis were lower (P = 0.016 for

254   *FASN* and P = 0.008 for *ME*), or tended to be lower (P = 0.097 for *SREBP1*) in pigs housed in

255   poor compared with good hygiene conditions whatever the RFI line. The relative expression of

256   the *LCN2* gene, a gene related to lipid transport, was also lower (P = 0.023) in pigs housed in

257   poor condition compared with those housed in good condition. Concerning the *TLR4* gene, its

258   expression was higher (P = 0.004) in pigs housed in poor than in pigs housed in good hygiene

259   conditions. Next, we found that the relative expression of the *GLUT4* gene (P = 0.062), *LEPTIN*

260   (P = 0.052) and *ADIPOR2* (P = 0.068) tended to be lower in pigs housed in poor compared with

261   pigs housed in good hygiene conditions. Then, the relative expressions of four other genes were

262   found to differ between RFI lines. The mRNA levels of *LCN2* was lower (P = 0.034) in HRFI

263 than in LRFI pigs. Among the genes encoding enzymes protecting cells from oxidative stress, we

264 identified that the mRNA levels of *GPX3* were higher (P < 0.001) in HRFI pigs than in LRFI

265 pigs. Similarly, the relative expression of the *TNF-α* (P = 0.049) and the *TLR2* (P = 0.033) genes

266 were higher in HRFI pigs than in LRFI pigs. Finally, the other genes studied were not

267 significantly impacted by hygiene conditions nor by the genetic line in the PRAT (Table 2 and

268 supplementary Table 1).

269 In SCAT (Table 3), the relative expression of only two genes were significantly affected by

270 housing conditions. The mRNA levels of *PPARG,* a gene involved in adipocyte differentiation,

271 decreased in pigs housed in poor compared with pigs housed in good hygiene condition (P =

272 0.049). Regarding the genes associated with the recognition of pathogens, it appeared that the

273 relative expression of *TLR4* decreased (P = 0.037) in pigs from poor compared with pigs from

274 good hygiene conditions whatever the RFI line. Next, we found that the relative expressions of

275 the *LCN2* gene, involved in lipid transport, were lower (P = 0.011) in HRFI pigs than in LRFI

276 pigs. Furthermore, the relative expression of the *GPX3* gene was higher (P < 0.001) in HRFI pigs

277 than in LRFI pigs. With respect to the relative gene expression of various adipokines and their

278 receptors, we have shown that the mRNA levels of *LEPTIN* (P = 0.022), *IGF1* (P = 0.030) and

279 *IL-10* (P = 0.016) were more expressed in HRFI pigs than in LRFI pigs whatever their housing

280 conditions. In the same way, the relative expression of the *TNF-α* gene tended to be increased (P

281 = 0.099) in HRFI pigs compared with LRFI pigs. Next, the relative expressions of the *ADIPOR1*

282 gene were lower (P = 0.043) in HRFI pigs than in LRFI pigs. Finally, the other genes studied

283 were not significantly impacted by hygiene conditions nor by the genetic line in the SCAT

284 (Supplementary table 2).

285

286 Adipogenic differentiation of SVF cells isolated from SCAT in response to TNF-α

287 Differentiation of SVF cells isolated from SCAT was determined on culture on D14, D18 and/or

288 D22 post-seeding. At D14, D18 and D22, adipocyte differentiation of cells was not impacted by

289 the genetic line, nor by the housing conditions of pigs (P > 0.05). Addition of TNF-α to the

290 medium tended to decrease or decreased adipocyte differentiation at D14 (P = 0.082), at D18 (P

291 = 0.046) and at D22 (P = 0.009) (Figures 1 and 2).

292

293 Whole blood cell response to LPS

294   In whole blood cells cultured in the absence or presence of LPS, the gene expression of *IL-1β*,

295   *IL-6*, *IL-10*, *IL-15*, *TNF-α*, *TGFβ1*, *TLR2*, *TLR4*, *GPX3*, *ADIPOR1*, *ADIPOR2* and *LEPR* were

296   not affected by hygiene of housing conditions (Table 4). The relative expression of the *GPX3*

297   gene was higher (P < 0.001) in HRFI than in LRFI pigs. In addition, the relative expressions of

298   the *IL-10* (P = 0.027), *TGFβ1* (P = 0.023) and *ADIPOR2* (P = 0.05) genes were lower in HRFI

299   than in LRFI pigs. The mRNA levels of *TGFβ1*, *ADIPOR1*, *ADIPOR2* and *LEPR* were not

300   influenced by the LPS stimulation in contrast to the other evaluated genes.

301

Discussion

303   In the current study, an experimental model of poor hygiene of housing conditions was used to

304   induce a systemic inflammation thereby activating the immune system of LRFI and HRFI

305   growing pigs, with LRFI pigs being more efficient than HRFI pigs, and modifying their

306   metabolism (Chatelet et al., 2018; Fraga et al., 2021). Higher concentrations of plasma

307   haptoglobin and of total IgG in animals placed in poor housing conditions confirmed that the

308   health challenge induced a moderate inflammatory state in both lines of RFI pigs (Chatelet et al.,

309   2018). Our major finding is that the responses measured in this study could explain, at least

310   partially, the better ability of LRFI pigs to cope with such a challenge as previously reported

311   (Chatelet et al., 2018). Indeed, the level of expression of the gene encoding the pro-inflammatory

312   cytokine TNF-α was lower in the two adipose tissues studied (PRAT and SCAT) in LRFI pigs

313   compared with HRFI pigs, whatever the hygiene conditions of housing. In addition, we observed

314   that blood immune cells from LRFI pigs exhibited a higher baseline level of anti-inflammatory

315   cytokine mRNA (*IL-10* and *TGFβ1*) compared with HRFI pigs. All of these data suggest that

316   LRFI pigs secrete fewer pro-inflammatory and higher anti-inflammatory cytokines compared

317   with HRFI pigs, which would help them to limit the deleterious effect of inflammation and

318   therefore to maintain better their growth performance in response to this health challenge

319   (Chatelet et al., 2018).

320

321   This study also indicates that the current challenge induced a depot-specific adipose tissue

322   response with a greater number of genes affected by the hygiene challenge in PRAT than in

323   SCAT. With the presence of Toll-like receptor 4 and 2, adipocytes and immune cells in adipose

324   tissue are able to respond to pathogen structures such as bacterial LPS (Lin et al., 2000;

325   Asadzadeh Manjili, Yousefi-Ahmadipour & Kazemi Arababadi, 2020). Thus, the finding of a

326   greater expression of the *TLR4* gene in PRAT of pigs housed in poor hygiene conditions may be

327   associated with an activation of TLR4 signaling pathways that are known to lead to the

328   production of IL-6 and TNF-α, two proinflammatory cytokines. This latter hypothesis is

329   consistent with a previous study showing that the TLR activation measured 6 h after a LPS

330   challenge in young pigs was associated with an overexpression of pro-inflammatory genes in

331   adipose tissue (Guo et al., 2015). Nevertheless, we failed to provide an increase in the

332   expressions of these two proinflammatory cytokines in response to the current challenge. This

333   suggests that PRAT either had a very limited inflammatory response to the hygiene challenge, or

334   we missed a short inflammatory response that may have occurred earlier in PRAT. This latter

335   hypothesis is supported by our previous findings (Chatelet et al., 2018) showing that the

336   inflammatory response of animals was greater three weeks after the onset of the health challenge

337   than after six weeks of exposure. In PRAT, we also observed a decrease in the expression of

338   genes involved in fatty acid and cholesterol synthesis (*FASN, SREBP1* and *ME*) and lipid

339   transport (*LCN2*). Similarly, *GLUT4* mRNA levels in PRAT tended to be lower in those pigs

340   suggesting a decrease in lipogenesis in this adipose depot. These results are consistent with the

341   finding of a lower mass of PRAT in pigs housed in poor conditions compared with pigs housed

342   in good hygiene conditions. We hypothesized that pigs may allocate more energy to cope with

343   the current challenge instead of storing it as fat in the PRAT. These changes were also associated

344   with greater activities of antioxidant enzymes in the PRAT of pigs housed in poor hygiene

345   conditions which supports the occurrence of oxidative stress (Sierzant et al., 2019). A more

346   extensive investigation of gene expressions (e.g. RNA-seq) is required to further support the idea

347   that PRAT is more responsive to the hygiene challenge than SCAT and to identify the main

348   biological processes affected by the hygiene challenge. Furthermore, other tissues/organs than

349   adipose tissue may respond to hygiene conditions. The study of skeletal muscle in these animals

350   at the end of the hygiene challenge is consistent with the presence of an inflammatory state

351   within this tissue (Quéméner et al., 2022). Indeed, pigs housed in poor hygiene conditions,

352   regardless of the RFI line, had a higher proportion of CD45^{+} hematopoietic cells than pigs

353   housed in standard conditions. Two recent studies from the same group based on the

354   investigation of transcriptomes of pigs differing in feed efficiency further support the role of
355   liver and muscle in the response to inflammatory stimuli (Horodyska et al., 2018, 2019a).

356

357   In SCAT, hygiene conditions decreased *TLR4* expression, and did not influence the expression of
358   genes of lipogenesis, which is consistent with the lack of change in the mass of this tissue at the
359   end of the challenge. In agreement with observations in PRAT, there was no influence of
360   hygiene conditions on the mRNA levels of *TNF-α* in SCAT. As TNF-α is mainly produced by
361   monocytes and macrophages, our recent finding of a similar proportion of hematopoietic cells,
362   identified as CD45^{+} cells, in SCAT of the four experimental groups (Quéméner et al., 2022)
363   further support the idea that an inflammatory response cannot be detected in SCAT at the end of
364   the challenge used in this study. Besides, the current findings further illustrate the differences in
365   the expression of genes between SCAT and PRAT which is located within the abdominal cavity.
366   In many species (Tchkonia et al., 2013) including pigs (Gondret et al., 2016), differences in
367   adipogenic factors, responses to hormones, metabolic properties or secretion of inflammatory
368   cytokines have been reported between SCAT and visceral adipose tissue. With respect to genes
369   related to inflammation and immune response, several examples related to human obesity,
370   demonstrate that visceral adipose tissue is more impacted than SCAT (Harman-Boehm et al.,
371   2007; Kranendonk et al., 2015). In these studies, some differences related to adipocyte sizes,
372   capillary densities and secreted amounts of adipokines have been reported. Therefore, during a
373   moderate but long-lasting inflammatory challenge, PRAT would be mobilized to support the
374   inflammatory response, while SCAT would be spared.

375

376   The observed decrease in the relative weight of PRAT in both RFI lines (Sierżant et al., 2019)
377   was associated with a reduction in the level of expression of the *LEPTIN* gene in PRAT of pigs
378   housed in poor hygiene conditions compared with those housed in good hygiene conditions. This
379   hormone is primarily produced and secreted by adipocytes, and is involved in the regulation of
380   feed intake and energy expenditure (Barb, 1999). The decrease in PRAT mass could contribute
381   to the decrease in leptinemia leading to resumption of feed intake. Nevertheless, we have shown
382   that the fasting plasma concentrations of leptin did not differ significantly between the four
383   investigated groups (Vincent et al., 2022). Since SCAT is the main adipose depot in pigs, our
384   results suggest that its contribution to leptinemia may be more significant than that of PRAT.

385   Thus, leptinemia, being a marker of fatty tissue quantity in the body (Kalina et al., 1999), could

386   be considered as a good marker of subcutaneous adiposity.

387

388   Further investigations were undertaken to determine whether the *in vitro* responses of adipose

389   tissue and blood cells to pro-inflammatory molecules were impacted by the hygiene challenge in

390   pigs of the two RFI lines. Our study clearly shows that TNF-α induced a reduction of adipogenic

391   differentiation of FSV cells isolated from SCAT as shown in previous studies (Petruschke &

392   Hauner, 1993; Xu, Sethi & Hotamisligil, 1999). It further indicates that the response to TNF-α

393   was similar in cells from the four experimental groups despite the findings of a higher proportion

394   of a population of mesenchymal stromal/stem cells (CD45⁻CD56⁻ cells) in SCAT from pigs

395   housed in poor hygiene conditions compared with pigs housed in good hygiene conditions

396   (Quéméner et al., 2022). Nevertheless, with the current available data, it is impossible to know

397   whether there are differences in differentiation ability between cell populations. Further studies

398   need to be performed, such as sorting SVF cell populations before their culture *in vitro*.

399   Similarly, there was no significant effect of hygiene conditions on the response of cultured blood

400   cells to LPS. We cannot exclude the hypothesis that we missed a change in the properties of cells

401   that may have occurred earlier.

402

403   Our study further shows that the expression of several genes in adipose tissues or in whole blood

404   cells differed between HRFI pigs and LRFI pigs regardless of their housing conditions. We

405   clearly found a higher expression of the gene encoding the GPX3 enzyme in PRAT, SCAT and

406   in blood cells in HRFI pigs compared with LRFI pigs. This enzyme is involved in the

407   detoxification of reactive oxygen species. This clear and systematic difference between the two

408   RFI lines have been observed previously in muscle and blood (Vincent et al., 2015; Jégou et al.,

409   2016). Because isolated adipocytes from HRFI pigs also produced more reactive oxygen species

410   (ROS) (Sierżant et al., 2019), we hypothesize that HRFI pigs produced more antioxidant

411   molecules in response to increased ROS production, potentially deleterious to cells, in order to

412   maintain their redox balance (Radak, Chung & Goto, 2005). However, the expression level of the

413   *GPX3* gene was not modified in response to the hygiene challenge in the two examined depots of

414   adipose tissue. This contrasts with our previous work (Sierżant et al., 2019) showing a clear

415   response of the anti-oxidant system of pigs, e.g. a greater activity of several antioxidant enzymes

416   (glutathione reductase, superoxide dismutase and catalase) in the PRAT of pigs housed in

417   degraded housing conditions. Thus, the present result for *GPX*3 indicates either that the response

418   to the hygiene inflammatory challenge did not occur at the transcriptional level for this enzyme,

419   or that GPX3 was only involved in the maintenance of the basal redox status but not in the

420   response to a challenge.

421

422   The present study shows that there were also line differences on the expression of genes related

423   to inflammation. Whole blood cells from HRFI pigs in culture expressed the same level of pro-

424   inflammatory cytokines (*IL-1β*, *IL-6*, *TNF-α*) and toll-like receptors (*TLR2* and *4*) as cells from

425   LRFI pigs, but had lower expression of *IL-10* and *TGF-β*, two cytokines involved in the down-

426   regulation of the inflammatory response compared with cells from LRFI pigs (Taylor et al.,

427   2006). With the current observations and a similar expression of *LEPTIN* and *ADIPOR1* genes in

428   blood cells of pigs from both RFI lines, we cannot determine whether the adipose-to-immune

429   tissue communication mediated these line effects. In PRAT, compared with LRFI pigs, HRFI

430   pigs expressed higher mRNA levels of *TLR2*, whose activation triggers a pro-inflammatory

431   signal, and of TNF-α, one of the main inflammatory cytokines mediating the metabolic effects of

432   inflammation (Gabler & Spurlock, 2008; Ajuwon, 2014;). Once again, effects were less clear-cut

433   in SCAT, where the gene expression increased for *IL-10* and tended to increase for *TNF-α* in

434   HRFI pigs compared with LRFI pigs. Altogether, these results suggest a greater pro-

435   inflammatory state in adipose tissues of the HFRI line in comparison with the LRFI line. This

436   difference between the two lines might play a role in the difference in feed efficiency, as

437   inflammation impairs adipose tissue accretion (Gabler & Spurlock, 2008), and in robustness to

438   environmental aggressions, as too much inflammation is counterproductive for resistance to

439   microbial infections and diseases resolution (Levy & Serhan, 2014). This is what was observed

440   with the present model of inflammation, since HRFI pigs had greater score of lung lesions and a

441   greater prevalence of pneumonia, whatever their hygiene environment (Chatelet et al, 2018).

Conclusions

443   The current study indicates that the impact of hygiene of housing on the expression of genes in

444   adipose tissues and on the features of SCAT adipose cells as well as whole blood cells in

445   response to TNF-α or LPS was similar in LRFI and HRFI pigs despite differences in the

446   expression of some genes like *GPX3* between the two lines. It further shows a depot-specific

447   response of adipose tissue to the challenge with a decrease in the expression of genes related to

448   fatty acid synthesis (*FASN* and *ME*) in PRAT but not in SCAT in response to the hygiene

449   challenge. A larger investigation of gene expressions is required to confirm this depot-specific

450   response. The complementary data obtained both *in vivo* and *in vitro* are not consistent with an

451   inflammatory response in adipose tissues and blood cells at the end of the challenge. Further

452   studies are needed to evaluate the hypothesis that adipose tissues and blood cells may have been

453   affected earlier.

454   **Data accessibility**

455   Data are available online:
456   https://data.inrae.fr/privateurl.xhtml?token=7f8daf50-f5cc-49fb-aaa3-0eb57b0a648c

457

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595

Primer sequences used for analysis of gene expression by qPCR

[1]Accession number in the National Center for Biotechnology Information database (

http://www.ncbi.nlm.nih.gov/gene) or Ensembl project database (

http://www.ensembl.org/Sus_scrofa/Info/Index) for pig sequences. [2]F = forward primer; [3]R =

reverse primer; [4]Genes used as reference for qPCR normalization.

1   Table 1: Primer sequences used for analysis of gene expression by qPCR.

Gene symbol	Gene name	Accession number[1]	Primer and probe sequences
ACOX1	*Acyl-CoA oxidase 1*	XM_021066020.1	F: TAGCCCTACTGTGACTTCCATCAA R: GCCAGTACTATCGCGTGATTTG
ADIPOQ	*Adiponectin*	NM_214370.1	F: GCTGTACTACTTCTCCTTCCACATCA R: CTGGTACTGGTCGTAGGTGAAGAGT
ADIPOR1	*Adiponectin receptor 1*	NM_001007193.1	F: GCCATGGAGAAGATGGAGGA R: AGCACGTCGTACGGGATGA
ADIPOR2	*Adiponectin receptor 2*	XM_021091197.1	F: TGTTCGCCACCCCTCAGTAT R: AATGATTCCACTCAGGCCCA
CEBPA	*CCAAT/enhancer binding protein alpha*	XM_003127015.4	F: GTGGACAAGAACAGCAACGA R: CTCCAGCACCTTCTGTTGAG
COX1	*Mitochondrial cytochrome c oxidase subunit 1*	XM_021064446.1	F: GAATAGTGGGCACTGCCTTGA R: GGGTTCCGGGCTGACCTA
COX3	*Mitochondrial cytochrome c oxidase subunit 3*	AF034253.1	F: CGTCCCATCCCTCGGTTTA R: GCCAGGTCGTGTGGATATTAGAGT
CS	*Citrate synthase*	XM_021091147.1	F: CCTTTCAGACCCCTACTTGTCCT R: CACATCTTTGCCGACTTCCTTC
DLK1	*Delta like non-canonical Notch ligand 1*	NM_001126101.2	F: CCCATGGAGCTGAATGCCT R: TTGCAAATGCACTGCCAGGG
ME	*Malic enzyme 1*	XM_001924333.5	F: TGGTGACTGATGGAGAACGTATTC R: CAGGATGACAGGCAGACATTCTT
FABP4	*Fatty acid binding protein 4*	NM_001002817.1	F: GGAAAGTCAAGAGCACCATAACCT R: ATTCCACCACCAACTTATCATCTACTATTT
FASN	*Fatty acid synthase*	NM_001099930.1	F: AGCCTAACTCCTCGCTGCAAT R: TCCTTGGAACCGTCTGTGTTC
GLUT4	*Glucose transporter 4*	NM_001128433.1	F: GGCAGCCCCTCATCATTG R: TCGAAGATGCTGGTTGAATAGTAGAA
GPX3	*Glutathione peroxidase 3*	NM_001115155.1	F: GCTTCCCCTGCAACCAATT R: GGACATACCTGAGAGTGGACAGAA
LIPE	*Lipase E hormone sensitive*	NM_214315.3	F: CAACTTGGTGCCCACAGAAGA R: GTCATGCAGTGTCAGGTACTTGAGA
IGF1	*Insulin-like growth factor 1*	NM_214256.1	F: GCTGGACCTGAGACCCTCTGT R: TACCCTGTGGGCTTGTTGAAAT
IGF2	*Insulin-like growth factor 2*	NM_213883.2	F: AGGGCATCCAAACCACAAAC R: GGGTTCAATTTTTGGTATGTAACTTG
IL-1β	*Interleukin 1beta*	NM_2140	F: GCCAGTCTTCATTGTTCAGGTTT

		55.1	R: TTGTCACCGTAGTTAGCCATCACT
IL-6	Interleukin 6	NM_001252429.1	F: CTGGCAGAAAACAACCTGAACC R: TGATTCTCATCAAGCAGGTCTCC
IL-10	Interleukin 10	NM_214041.1	F: TGAGAACAGCTGCATCCACTTC R: TCTGGTCCTTCGTTTGAAAGAAA
IL-15	Interleukin 15	XM_021100480.1	F: TGCATCCAGTGCTACTTGTGTT R: TTAGGAAGACCTGCACTGATACAG
LCN2	Lipocalin 2	XM_021088538.1	F: TCGCAATCGACCAGTGCAT R: TGGGCAAAGGCTGAAGACAT
LEPTIN	Leptin	XM_021078503.1	F: GTTGAAGCCGTGCCCATCT R: CTGATCCTGGTGACAATCGTCTT
LEPR	Leptin Receptor	NM_001024587.1	F: CAGTCGCTCAGTGCTTATCCT R: GGAAGGGATTCTGAGCCATT
PPARG	Peroxisome proliferator activated receptor gamma	XM_005669788.3	F: ATTCCCGAGAGCTGATCCAA R: TGGAACCCCGAGGCTTTAT
SOD2	Superoxide dismutase 2	NM_214127.2	F: GCGCTGAAAAAGGGTGATGT R: ACCGTTAGGGCTCAGATTTGTC
SREBP1	Sterol regulatory element binding transcription factor 1	XM_021066226.1	F: CGGACGGCTCACAATGC R: GCAAGACGGCGGATTTATTC
TGFβ1	Transforming growth factor beta 1	XM_021093503.1	F: AGCGGCAACCAAATCTATGATAA R: CGACGTGTTGAACAGCATATATAAGC
TLR2	Toll like receptor 2	XM_005653577.3	F: CCCAGCACAGTGATGAAAAATT R: TCCGTAAAACTTGCGTCAGTGA
TLR4	Toll like receptor 4	NM_001113039.2	F: AACATCCCCACATCAGTCAAGAT R: CCCTGATATGCATCATCGTCAA
TNF-α	Tumor necrosis factor alpha	NM_214022.1	F: GGTTATCGGCCCCCAGAA R: TGGGCGACGGGCTTATC
UCP3	Uncoupling protein 3	NM_214049.1	F: AAGTACAGCGGGACGATGGA R: TGTTGGGCAGAATTCCTTTCC
B2M	Beta-2-microglobulin	NM_213978	F : AAACGGAAAGCCAAATTACC R : ATCCACAGCGTTAGGAGTGA
PPIA	Peptidylprolyl isomerase A	XM_021078519.1	F: AGCACTGGGGAGAAAGGATT R: AAAACTGGGAACCGTTTGTG
TBP1	TATA box binding protein	DN110073	F : AACAGTTCAGTAGTTATGAGCCAGA R : AGATGTTCTCAAACGCTTCG
YWHAZ	Tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein zeta	XM_005662949.2	F: ATGCAACCAACACATCCTATC R: GCATTATTAGCGTGCTGTCTT

3   [1]Accession number in the National Center for Biotechnology Information database
4   (http://www.ncbi.nlm.nih.gov/gene) or Ensembl project database
5   (http://www.ensembl.org/Sus_scrofa/Info/Index) for pig sequences.
6   [2]F = forward primer; [3]R = reverse primer; [4]Genes used as reference for qPCR normalization.
7

Relative expression of genes in perirenal adipose tissue 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 = 7-10 pigs/experimental group). [1]Probability values for the effect of hygiene conditions (Hyg), genetic lines (Line), and the Hyg x Line (H x L) interaction. Boldface highlights significant differences (P ≤ 0.05) and italicized character shows a trend (0.05 < P < 0.10).

1   Table 2: Relative expression of genes in perirenal adipose tissue of low (LRFI) and high (HRFI)
2   residual feed intake pigs housed in good or poor hygiene conditions for six weeks.

Genes	Good		Poor		P-values[1]		
	LRFI	HRFI	LRFI	HRFI	Hyg	Line	H x L
Adipocyte differentiation							
PPARG	0.52 ± 0.07	0.37 ± 0.10	0.35 ± 0.07	0.46 ± 0.13	0.639	0.867	0.168
Lipid metabolism							
FASN	0.44 ± 0.08	0.34 ± 0.07	0.29 ± 0.04	0.16 ± 0.03	**0.016**	*0.076*	0.880
SREBP1	0.59 ± 0.05	0.68 ± 0.08	0.57 ± 0.04	0.50 ± 0.08	*0.097*	0.893	0.203
ME	0.48 ± 0.08	0.36 ± 0.06	0.29 ± 0.03	0.20 ± 0.03	**0.008**	*0.091*	0.791
Lipid transport							
LCN2	0.36 ± 0.06	0.22 ± 0.04	0.21 ± 0.03	0.13 ± 0.02	**0.023**	**0.034**	0.521
Carbohydrate metabolism							
GLUT4	0.45 ± 0.07	0.37 ± 0.05	0.33 ± 0.05	0.27 ± 0.03	*0.062*	0.250	0.924
Mitochondrial metabolism							
COX1	0.60 ± 0.03	0.65 ± 0.03	0.72 ± 0.05	0.62 ± 0.08	0.379	0.628	0.150
Oxidative stress							
GPX3	0.25 ± 0.01	0.45 ± 0.04	0.23 ± 0.02	0.57 ± 0.09	0.246	**< 0.001**	0.131
Adipokines							
ADIPOQ	0.60 ± 0.03	0.63 ± 0.04	0.59 ± 0.03	0.59 ± 0.07	0.544	0.765	0.702
ADIPOR1	0.66 ± 0.03	0.61 ± 0.03	0.68 ± 0.04	0.63 ± 0.03	0.680	0.221	0.922
ADIPOR2	0.72 ± 0.05	0.63 ± 0.05	0.62 ± 0.04	0.53 ± 0.05	*0.068*	0.110	0.993
LEPTIN	0.44 ± 0.09	0.37 ± 0.08	0.26 ± 0.11	0.17 ± 0.05	*0.052*	0.385	0.950
IGF1	0.63 ± 0.04	0.64 ± 0.06	0.63 ± 0.03	0.64 ± 0.04	0.943	0.818	0.943
TNF-α	0.37 ± 0.04	0.53 ± 0.11	0.31 ± 0.05	0.41 ± 0.06	0.182	**0.049**	0.612
IL-10	0.23 ± 0.02	0.36 ± 0.11	0.33 ± 0.06	0.51 ± 0.18	0.261	0.157	0.797
Pathogen recognition							
TLR2	0.47 ± 0.06	0.61 ± 0.10	0.45 ± 0.05	0.62 ± 0.07	0.872	**0.033**	0.808
TLR4	0.38 ± 0.04	0.44 ± 0.07	0.63 ± 0.08	0.61 ± 0.05	**0.004**	0.831	0.579

3   Values are means ± SEM (n = 7-10 pigs/experimental group). [1]Probability values for the effect
4   of hygiene conditions (Hyg), genetic lines (Line), and the Hyg x Line (H x L) interaction.
5   Boldface highlights significant differences (P ≤ 0.05) and italicized character shows a trend (0.05
6   < P ≤ 0.10).

Relative expression of genes in subcutaneous adipose tissue 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 = 5-10 pigs/experimental group). [1]Probability values for the effect of hygiene conditions (Hyg), genetic lines (Line), and the Hyg x Line (H x L) interaction. Boldface highlights significant differences (P ≤ 0.05) and italicized character shows a trend (0.05 < P < 0.10).

1 Table 3: Relative expression of genes in subcutaneous adipose tissue of low (LRFI) and high
2 (HRFI) residual feed intake pigs housed in good or poor hygiene conditions for six weeks.

	Good		Poor		*P-values[1]*		
Genes	**LRFI**	**HRFI**	**LRFI**	**HRFI**	**Hyg**	**Line**	**H x L**
Adipocyte differentiation							
PPARG	0.60 ± 0.07	0.57 ± 0.09	0.44 ± 0.08	0.38 ± 0.12	**0.049**	0.611	0.849
Lipid metabolism							
FASN	0.48 ± 0.08	0.40 ± 0.10	0.41 ± 0.05	0.23 ± 0.04	0.132	*0.093*	0.504
SREBP1	0.58 ± 0.05	0.65 ± 0.10	0.66 ± 0.05	0.59 ± 0.05	0.889	0.981	0.276
ME	0.46 ± 0.07	0.35 ± 0.06	0.37 ± 0.04	0.29 ± 0.06	0.174	0.102	0.824
Lipid transport							
LCN2	0.44 ± 0.06	0.31 ± 0.03	0.42 ± 0.08	0.24 ± 0.02	0.443	**0.011**	0.687
Carbohydrate metabolism							
GLUT4	0.54 ± 0.05	0.51 ± 0.08	0.51 ± 0.04	0.54 ± 0.05	0.976	0.955	0.642
Mitochondrial metabolism							
COX1	0.49 ± 0.04	0.51 ± 0.04	0.65 ± 0.05	0.46 ± 0.05	0.299	*0.082*	**0.039**
Oxidative stress							
GPX3	0.32 ± 0.03	0.54 ± 0.09	0.33 ± 0.03	0.71 ± 0.10	0.149	**<0.001**	0.190
Adipokines							
ADIPOQ	0.56 ± 0.03	0.58 ± 0.04	0.61 ± 0.04	0.57 ± 0.08	0.655	0.771	0.525
ADIPOR1	0.69 ± 0.03	0.65 ± 0.03	0.80 ± 0.04	0.68 ± 0.05	*0.078*	**0.043**	0.294
ADIPOR2	0.73 ± 0.05	0.70 ± 0.05	0.73 ± 0.03	0.65 ± 0.06	0.623	0.265	0.573
LEPTIN	0.29 ± 0.05	0.37 ± 0.08	0.18 ± 0.04	0.46 ± 0.12	0.879	**0.022**	0.217
IGF1	0.70 ± 0.05	0.76 ± 0.05	0.67 ± 0.02	0.82 ± 0.05	0.690	**0.030**	0.328
TNF-α	0.51 ± 0.08	0.66 ± 0.06	0.51 ± 0.06	0.64 ± 0.12	0.901	*0.099*	0.878
IL-10	0.36 ± 0.04	0.71 ± 0.13	0.32 ± 0.04	0.44 ± 0.16	0.118	**0.016**	0.216
Pathogen recognition							
TLR2	0.44 ± 0.06	0.53 ± 0.05	0.45 ± 0.04	0.47 ± 0.06	0.653	0.323	0.523

| *TLR4* | 0.56 ± 0.05 | 0.58 ± 0.07 | 0.44 ± 0.07 | 0.41 ± 0.07 | **0.037** | 0.928 | 0.794 |

4 Values are means ± SEM (n = 5-10 pigs/experimental group). [1]Probability values for the effect
5 of hygiene conditions (Hyg), genetic lines (Line), and the Hyg x Line (H x L) interaction.
6 Boldface highlights significant differences (P ≤ 0.05) and italicized character shows a trend (0.05
7 < P ≤ 0.10).
8

Relative expression of genes in whole blood cells cultured in the absence (-) or presence (+) of LPS and obtained from 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). [1]Probability values for the effect of the LPS treatment (LPS) and the genetic lines (Line). There was no significant effect of hygiene conditions and no significant interactions. Boldface highlights significant differences (P ≤ 0.05) and italicized character shows a trend (0.05 < P < 0.10).

1 Table 4: Relative expression of genes in whole blood cells cultured in the absence (-) or presence
2 (+) of LPS and obtained from low (LRFI) and high (HRFI) residual feed intake pigs housed in
3 good or poor hygiene conditions for six weeks.

		Good		Poor			
Genes	LPS	LRFI	HRFI	LRFI	HRFI	LPS	Line
GPX3	-	0.76 ± 0.27	2.86 ± 1.28	0.46 ± 0.07	3.66 ± 1.32	0.011	<0.001
	+	0.34 ± 0.06	1.33 ± 0.38	0.26 ± 0.04	1.65 ± 0.42		
IL-10	-	0.29 ± 0.07	0.21 ± 0.05	0.35 ± 0.08	0.28 ± 0.07	<0.001	0.027
	+	3.23 ± 0.38	2.35 ± 0.49	3.10 ± 0.17	2.41 ± 0.32		
TGFβ1	-	1.62 ± 0.26	1.15 ± 0.20	1.61 ± 0.20	1.37 ± 0.21	0.51	0.023
	+	1.64 ± 0.18	1.23 ± 0.26	1.79 ± 0.19	1.50 ± 0.16		
IL-6	-	0.93 ± 0.92	0.01 ± 0.005	0.04 ± 0.02	0.02 ± 0.005	0.024	0.357
	+	0.34 ± 0.10	1.12 ± 0.37	2.11 ± 0.83	0.89 ± 0.35		
TNF-α	-	1.06 ± 0.42	0.60 ± 0.18	0.66 ± 0.05	0.61 ± 0.14	<0.001	0.753
	+	1.30 ± 0.18	2.87 ± 0.84	2.50 ± 0.97	1.93 ± 0.38		
IL-1β	-	1.08 ± 1.05	0.02 ± 0.006	0.04 ± 0.003	0.03 ± 0.003	<0.001	0.514
	+	1.30 ± 0.32	2.36 ± 0.84	2.59 ± 0.80	1.44 ± 0.46		
IL-15	-	3.29 ± 1.38	2.54 ± 0.64	3.54 ± 0.69	2.46 ± 0.28	0.001	0.400
	+	1.42 ± 0.24	1.57 ± 0.43	1.21 ± 0.22	1.32 ± 0.31		
TLR2	-	0.81 ± 0.24	0.53 ± 0.13	0.79 ± 0.15	0.75 ± 0.22	<0.001	0.254
	+	1.44 ± 0.28	1.13 ± 0.13	1.30 ± 0.20	1.24 ± 0.19		
TLR4	-	0.65 ± 0.18	0.41 ± 0.12	0.76 ± 0.18	0.53 ± 0.14	0.024	0.109
	+	1.00 ± 0.25	0.82 ± 0.15	1.03 ± 0.21	0.78 ± 0.16		
ADIPOR1	-	0.97 ± 0.25	1.08 ± 0.29	0.93 ± 0.23	1.38 ± 0.29	0.948	0.155
	+	1.14 ± 0.18	1.28 ± 0.34	0.85 ± 0.15	1.13 ± 0.20		
ADIPOR2	-	1.10 ± 0.23	0.84 ± 0.20	0.99 ± 0.17	0.80 ± 0.21	0.257	0.05
	+	0.92 ± 0.12	0.56 ± 0.07	0.93 ± 0.13	0.80 ± 0.15		
LEPR	-	1.26 ± 0.45	1.07 ± 0.42	1.10 ± 0.24	1.56 ± 0.37	0.988	0.198

| | + | 0.55 ± 0.14 | 0.85 ± 0.22 | 0.86 ± 0.17 | 2.75 ± 1.64 | |

5 Values are means ± SEM (n = 5-9 pigs/experimental group). [1]Probability values for the effect of
6 the LPS treatment (LPS) and the genetic lines (Line). There was no significant effect of hygiene
7 conditions and no significant interactions. Boldface highlights significant differences ($P \leq 0.05$)
8 and italicized character shows a trend ($0.05 < P \leq 0.10$).

Figure 1

Photomicrographs of cultured cells at day 14 post seeding

Cells from the stromal vascular fraction were isolated from subcutaneous adipose tissue of pigs at the end of the hygiene challenge and were cultured in adipogenic medium in the absence (A) or presence (B) of 10 ng/mL TNF-α (X40, white bar stands for 10 μm).
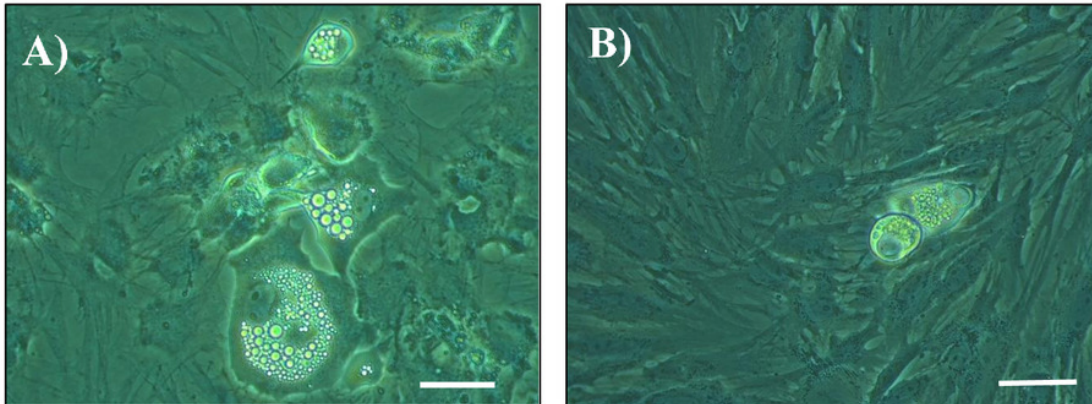
Figure 2

Adipocyte differentiation of cells at Day 14, Day 18 and Day 22 post-seeding

Cells from the stromal vascular fraction were isolated from subcutaneous adipose tissue of high (HRFI, white bar) and low (LRFI, black bar) residual feed intake pigs housed in good or poor hygiene conditions in the absence or presence of TNF-α. Adipocyte differentiation are expressed as means ± SEM (n = 3-5 pigs/experimental group). Boldface highlights significant differences (P ≤ 0.05) and italicized character shows a trend (0.05 < P < 0.10). All interactions are greater than 0.1 at Days 14, 18 and 22.

A

Day 14

TNF-α: *0.082*
Hyg: 0.722
Line: 0.922

B

Day 18

TNF-α: **0.046**
Hyg: 0.459
Line: 0.882

C

Day 22

TNF-α: **0.009**
Hyg: 0.128
Line: *0.087*

□HRFI   ■LRFI