Martinez Alvaro, M.; Agha, SA.; Blasco Mateu, A.; Hernández, P. (2017). Muscle metabolism in two rabbit lines divergently selected for intramuscular fat. Journal of Animal Science. 95(6):2576-2584. doi:10.2527/jas2017.1371

Running head: metabolism of intramuscular fat

Muscle lipid metabolism in two rabbit lines divergently selected for intramuscular fat[1]

M. Martínez-Álvaro*, S. Agha†, A. Blasco* and P. Hernández[2]*

*Institute for Animal Science and Technology, Universitat Politècnica de València. 46022 Valencia, Spain.

†Animal Production Department, Faculty of Agriculture, Ain Shams University, Cairo, Egypt.

[1]This work was supported by project AGL2014-55921-C2-01-P from the Spanish National Research Plan. M. Martínez-Álvaro acknowledges a FPI grant (BES-2012-052655) from the Economy Ministry of Spain. The authors thank Federico Pardo for their technical assistance.

[2] Corresponding author: Hernández, Pilar. E-mail: phernan@dca.upv.es

ABSTRACT

A divergent selection experiment for intramuscular fat (IMF) of Longissismus dorsi muscle at 9 wk of age was performed in rabbits. The objective of this work is to compare the lipid metabolism in muscles and fat tissues of the high-IMF and low-IMF lines. Lipogenic, catabolic and lipolytic activities were studied in two muscles with different oxidative pattern (Longissimus dorsi and Semimembranosus proprius) and perirenal fat depot at two ages, 9 wk and 13 wk. Besides, adipocytes were characterized in Semimembranosus proprius muscle and perirenal fat. In the fifth generation, direct response to selection was 0.26 g / 100 g. Lines showed differences in their lipogenic activities of muscles and fat tissues at 13 wk, but not at 9 wk. High-IMF line showed greater lipogenic activities in Longissimus dorsi and greater G6PDH and FAS activities in Semimembranosus proprius and perirenal fat than low-IMF line. However, in perirenal fat, EM activity was greater in the low-IMF line. No differences between lines were found in catabolic or lipolytic activities of muscles. Regarding adipocytes characteristics, high-IMF line showed larger adipocytes in Semimembranosus proprius and perirenal fat depot tissues compared to the low-IMF line, but no differences between lines were observed in the number of adipocytes.

Key words: intramuscular fat, lipid metabolism, rabbits.

INTRODUCTION

Intramuscular fat (**IMF**) plays an essential role in meat quality, affecting organoleptic and technologic meat properties (Wood et al. 2008). Intramuscular fat content can be easily modified by genetic selection due to its high heritability (Martínez-Álvaro et al. 2016) and variability. However, only three selection experiments for IMF have been published (Schwab et al. 2009 in pigs, Sapp et al. 2002 in cattle and Zhao et al. 2007 in chickens). In the Polytechnic University of Valencia we are performing a divergent selection experiment for IMF of Longissimus dorsi muscle in rabbits (Martínez-Álvaro et al., 2016). Rabbit is an excellent animal model for genetic experiments due to its short generation interval and the low cost of its carcasses.

An increased IMF content has been related to greater lipogenic rate in muscle and changes in catabolic activities in several species, rabbits (Zomeño et al. 2010 and Hernández et al. 2008), pigs (Mourot and Kouba, 1998, 1999 and Gondret and Lebret, 2007) and cattle (Bonnet et al. 2007 and Hocquette et al. 2012). Besides, IMF differences have been ascribed to differences in the size and/or number of intramuscular adipocytes (Hauser et al. 1997 and Damon et al. 2006 in pigs). In our divergent selection experiment lipid metabolism can be compared in rabbit lines with the same genetic origin and environment, differing only in IMF and correlated traits. None of the previous selection experiments for IMF analyzed the lipid metabolism of their lines.

Generally, commercial carcass of rabbits varies between countries from 9 wk to 13 wk of age, showing greater IMF content at the elder age. Animals from both ages were used in our experiment.

Selection for IMF of Longissimus dorsi could affect in different ways the lipid metabolism of muscles having other oxidative patterns and fat depots. The objective of this work is to compare the lipid metabolism and adipocytes cellularity of muscles with diverse oxidative pattern and fat tissues of two rabbit lines divergently selected for IMF.

MATERIALS AND METHODS

Animals

This study was performed with rabbits from the fifth generation of a divergent selection experiment for IMF of Longissimus dorsi muscle at 9 wk of age. Two rabbits (a male and a female) from the first parity of each doe were evaluated for IMF content, and the average between these two values was calculated. Then, all dams were ranked according to this average, and selection for high or low IMF was performed on rabbits from the second parity. All females of the approximately 20% best dams were selected for next generation. As each sire was mated with five dams, only one male of its best dam was selected. This selection within male family was performed in order to reduce inbreeding. Normally, the first parity was used to collect the IMF data and the second parity to select the rabbits for next generation, although exceptionally some IMF measurements were made on the second or third parity. More details of this experiment can be found in Martínez-Álvaro et al. (2016). Litters were homogenised at birth up to 9 kits per litter. Rabbits were reared collectively from weaning to slaughter and were fed ad libitum with a commercial diet. They were under a constant photoperiod of 16:8 h and controlled ventilation.

Direct response to selection was estimated using 202 rabbits (100 from the high-IMF line and 102 from the low-IMF line), slaughtered at 9 wk using electrical stunning and exsanguination. After slaughter, carcasses were chilled for 24h at 4ºC. Then,

Longissimus dorsi muscle was excised, minced, freeze-dried and scanned with Near Infrarred Spectrophotometry (model 5000, FOSS NIRSystems INC., Hilleroed, Denmark) to measure IMF, using the prediction equations developed by Zomeño et al. (2011 and 2012). Intramuscular fat was expressed as g/100 g of muscle on a fresh basis.

Enzyme activities were measured in an additional sample of 110 rabbits, 62 slaughtered at 9 wk (32 from high-IMF and 30 from low-IMF line) and 48 slaughtered at 13 wk (24 per line). Adipocytes were characterized in another additional sample of 45 rabbits, 23 slaughtered at 9 wk (10 from high-IMF and 13 from low-IMF line) and 22 slaughtered at 13 wk (10 from high-IMF and 12 from low-IMF line). Immediately after slaughter, hot carcass weight was registered according to the norms proposed by the World Rabbit Science Association (Blasco and Ouhayoun, 1996). Longissimus dorsi and Semimembranosus proprius muscles and perirenal fat depot were excised and weighted. Samples of the three tissues were frozen in liquid nitrogen and stored at −80°C for enzymatic assays.

All experimental procedures involving animals were approved by the Universitat Politècnica de València Research Ethics Committee, according to Council Directives 98/58/EC (European Economic Community, 1998) and 2010/63/EU (European Commision Directive, 2010).

Measurement of enzyme activities

Activities of lipogenic enzymes glucose-6-phosphate dehydrogenase (**G6PDH**), malic enzyme (**EM**) and fatty acid synthase (**FAS**) were measured in Longissimus dorsi and Semimembranosus proprius muscles and perirenal fat. A weighted quantity of tissue (1 g for Longissimus dorsi and perirenal fat and 0.5 g for Semimembranosus proprius) was homogenized in a volume of ice-cold 0.25 M sucrose solution containing

1mM dithiothreitol and 1mM EDTA (2.5 ml for muscles and 5 ml for perirenal fat). Homogenates were centrifuged at 12,000 x *g* for 1 h at 4 ºC and cytosolic supernatants were filtered through glass wool and collected for enzyme assays. Activities were assessed at 37 ºC using a spectrophotometer (model UV-1601, Shimadzu Co, Tokyo, Japan) at 340 nm according to the methods described by Fitch et al. (1959) for G6PDH, Hsu and Lardy (1969) for EM and Chang et al. (1967) for FAS. Enzyme activities were expressed in nmol of NADPH produced (G6PDH and EM) or oxidized (FAS) per min and per g of fresh tissue.

Activities of catabolic enzymes β-hydroxyacyl-CoA dehydrogenase (**HAD**), citrate synthase (**CS**) and lactate dehydrogenase (**LDH**) were determined in Longissimus dorsi and Semimembranosus proprius muscles. Samples of 0.2 g of tissue were homogeneized in 50 vol (wt/vol) of ice-cold 0.1 M phosphate buffer (pH 7.5) 2mM EDTA. Homogenates were centrifuged at 6,000 x *g* for 15 min at 4 ºC and the resulting cytosolic supernatants were filtered as described above. Activities were assessed at 30 ºC in a spectrophotometric analyzer Fluostar Galaxy (BMG Lab Technologies, Offenburg, Germany) at 340 nm for HAD and LDH or at 405 nm for CS according to the methods described by Bass et al. (1969), Srere (1969) and Bergmeyer and Bernt (1974), respectively. Enzyme activities were expressed as µmol of NADH (HAD, LDH) or of mercaptide ion (CS) released per min and per g of fresh tissue.

Activities of lipolytic enzymes acid lipase (**AL**), neutral lipase (**NL**) and acid phospholipase (**APL**) were assayed on Longissimus dorsi muscle, according to the method described by Hernández et al. (1999) using 4-methylumbelliferyloleate as fluorescent substrate. Lipolytic activity was measured only in Longissimus dorsi because of the lack of enough Semimembranosus proprius sample remaining after other analyses. A sample of 4 g was homogenized in 20 ml of 50 mM phosphate buffer (pH

7.5) containing 5 mM ethylene glycol tetraacetic acid. The homogenate was centrifuged at a 10000 x *g* for 20 min at 4 ºC and the resulting supernatant was filtered as described above. Reaction mixtures of lipase assays with fluometric substrates were incubated at 37 ºC for 20 min. The fluorescence was measured at an excitation wavelength of 460 nm using a Fluostar Galaxy fluometer (BMG Lab Technologies, Offenburg, Germany). Enzyme activities were expressed as µmol of substrate hydrolyzed per h and per g of fresh tissue.

Soluble protein was determined in muscles supernatant using the bicinchoninic acid (BCA) Protein Assay Kit (Smith et al. 1985) provided by Pierce (Rockford, Illinois, United States) and enzyme activities were also expressed in a soluble-protein content basis.

Characterization of adipocytes

Samples of 0.5 g of Semimembranosus proprius muscle and perirenal fat were collected immediately after slaughter and placed in tubes with 10 ml of Tyrode's solution (0.15 M NaCl; 6 mM KCl; 2mM $CaCl_2$; 6 mM glucose, 2 mM $NaHCO_3$, pH 7.6) at 39 ºC for adipocytes cellularity measurements. We could not characterize the adipocytes in Longissimus dorsi muscle because of the low size and number of the fat cells. Samples underwent digestion with collagenase (Rodbell, 1964). Approximately 100 mg of each sample was digested with collagenase type 2 (0.1 mg fat and 0.15 mg for muscle), 4 mg of bovine serum albumin and 100 µl of T199 medium at 39 °C during 1 h for fat or 2 h for muscle. After digestion, a drop of the superficial phase was taken to prepare slides for microscope examination. Images obtained with the microscope were digitized and analyzed using the image analysis software ImageJ (U. S. National Institutes of Health, Bethesda, Maryland, USA). Adipocytes diameter was determined

on 300 and 50 cells for fat and muscle, respectively, and the average diameter was calculated. Adipocytes with diameters under 20 µm and over 250 µm were excluded from the count. Lipid content was determined in the resting tissue by ether extraction (Soxtec 1043 extraction unit, Tecator, Höganäs, Sweden) and was expressed as g/100 g of fresh tissue. Number of adipocytes per g of tissue was calculated dividing the lipid content in a g of sample by the lipid content of one adipocyte (assuming a lipid density value of 0.915 g/ml and cells to be spherical in shape). Total number of adipocytes was calculated multiplying the number of adipocytes per g of tissue by the weight of the tissue.

Statistical analyses

Descriptive statistics of the traits at 9 and 13 wk of age were performed after correcting data by line, sex and parity order effects. Since data of the selection trait was collected during a long period of time, the effect of month-season was included in the analysis. For enzymatic activities and adipocytes characteristics, the effect of parity order was not included in the analysis, since most of the animals came from the same parity. For adipocytes characteristics, records at 9 and 13 wk were analyzed together, correcting data by the effect of age.

Effect of selection was estimated as the differences between high-IMF and low-IMF lines at 9 and 13 wk. Models included fixed effects of line, sex, month-season and parity order as indicated before, and common litter as a random effect. For adipocytes characteristics differences between lines were estimated with the same model including the fixed effect of age.

A Bayesian analysis was performed. Common litter effect and residuals of the models were assumed to be independently normally distributed. Bounded flat priors

were assumed for all fixed effects and variances. Marginal posterior distributions were estimated using Gibbs Sampling, testing the convergence for each chain with the Z criterion of Geweke, and Monte Carlo sampling errors were computed using time-series procedures (Sorensen and Gianola, 2002). Chains of 60,000 samples with a burn-in period of 10,000 were used. The programme "Rabbit" developed by the Institute for Animal Science and Technology (Valencia, Spain) was used for the analysis. The parameters obtained from the marginal posterior distributions of the differences between lines were the median (**D**), the highest posterior density region at 95% (**$HPD_{95\%}$**) and the probability of the lines being different (probability of the difference being greater than zero when D is positive, or lower than zero when D is negative) (**P_0**). More details of these features can be found in Blasco (2001, 2005).

RESULTS

Direct response to selection and correlated responses in carcass traits

In the fifth generation, the mean of the selection trait, IMF of Longissimus dorsi muscle at 9 wk, was 1.03 g of IMF/100 g of muscle with a SD of 0.15. Direct response to selection was 0.26 (P_0 = 1.00) with a $HPD_{95\%}$ from 0.21 to 0.31.

Table 1 shows descriptive statistics and differences between lines in carcass traits at 9 and 13 wk of age. Perirenal fat weight was greater in high-IMF than in low-IMF line at 13 wk of age (P_0 = 1.00) but we did not observed differences between lines at 9 wk. No differences between lines were observed in hot carcass and muscles weights at any age, except for hot carcass weight at 13 wk that was greater in the low-IMF than in the high-IMF line (P_0 = 0.91).

211 ***Lipogenic enzyme activities in muscles and perirenal fat***

212 Table 2 shows descriptive statistics and differences between lines in lipogenic
213 activities in muscles and perirenal fat at 9 and 13 wk in nmol / min and g of tissue.
214 Semimembranosus proprius muscle showed greater G6PDH and FAS activities and less
215 EM activity than Longissimus dorsi at both 9 and 13 wk. However, EM activity was
216 greater in Semimembranous proprius when the results were expressed in a soluble-
217 protein basis, due to the low protein content of this muscle (data not shown). Perirenal
218 fat tissue showed greater G6PDH and FAS activities and lower EM activity than
219 muscles at both ages.

220 All the lipogenic activities were greater at 13 than at 9 wk in the three tissues.
221 The differences between 13 and 9 wk of age in nmol / min and g of tissue were 11.6 for
222 G6PDH (P_0 = 0.96), 265 for EM (P_0 = 1.00) and 4.77 for FAS (P_0 = 0.99) in
223 Longissimus dorsi muscle; 139 for G6PDH (P_0 = 1.00), 38.2 for EM (P_0 = 0.96) and
224 24.8 for FAS (P_0 = 1.00) in Semimembranosus proprius muscle and 175 for G6PDH (P_0
225 = 1.00), 72.5 for EM (P_0 = 1.00) and 52.6 for FAS (P_0 = 0.99) in perirenal fat.

226 Lines showed differences in their lipogenic activities at 13 wk but not at 9 wk.
227 At 13 wk, high-IMF line showed greater G6PDH (P_0 = 1.00), EM (P_0 = 0.93) and FAS
228 (P_0 = 0.90) activities in Longissimus dorsi, and greater G6PDH (P_0 = 0.98) and FAS (P_0
229 = 0.95) activities in Semimembranosus proprius than low-IMF line, whereas there was a
230 low evidence for the difference between lines in EM activity in this muscle (P_0 = 0.82).
231 Similar results were obtained when activities were expressed in a soluble-protein basis
232 (data not shown). In perirenal fat, high-IMF had greater G6PDH (P_0 = 0.91) and FAS
233 (P_0 = 0.96) activities, but lower EM activity than low-IMF line (P_0 = 0.90). At 9 wk, we
234 did not observe differences between lines in lipogenic activities, except for EM activity
235 in Semimembranosus proprius that was greater in the low-IMF line (P_0 = 0.98).

236 ***Catabolic enzyme activities in muscles***

237 Table 3 shows descriptive statistics and differences between lines in oxidative,
238 glycolytic (both expressed in µmol/min and g of tissue) and lipolytic (in µmol/h and g
239 of tissue) activities in Longissimus dorsi and Semimembranosus proprius muscles at 9
240 and 13 wk. Longissimus dorsi muscle had greater LDH activity whereas
241 Semimembranosus proprius showed greater HAD and CS activities, according to their
242 different oxidative pattern. Similar results were obtained when activities were expressed
243 in a soluble-protein basis (data not shown). Oxidative and glycolytic activities were
244 similar at both ages, except for HAD activity in Semimembranosus proprius that
245 decreased with age; the difference between ages (13 wk – 9 wk) in HAD activity was -
246 0.22 µmol/min and g of tissue (P_0 = 0.93). Lipolytic activities in Longissimus dorsi
247 decreased with age (P_0 = 1.00); the differences between ages (13 wk – 9 wk) in µmol/h
248 and g of tissue were -0.11 for LA, -0.89 for LN and -0.07 for PLA.

249 We almost did not find differences between lines in the catabolic activities of
250 muscles. Few differences between lines were observed in oxidative activities, although
251 they were not consistent between muscles or ages. High-IMF line showed greater HAD
252 activity in Longissimus dorsi only at 9 wk (P_0 = 0.96), whereas low-IMF line showed
253 greater CS activity in Semimembranosus proprius only at 13 wk (P_0 = 0.90). We did not
254 observe differences between lines in LDH or lipolytic activities. Results did not change
255 when activities were expressed in a soluble-protein basis (data not shown).

256 ***Adipocytes characteristics in Semimembranosus proprius muscle and perirenal fat***

257 Table 4 shows descriptive statistics and differences between lines in the
258 adipocytes characteristics of Semimembranosus proprius muscle and perirenal fat. As it
259 was expected, the size and the number of the adipocytes were greater in the fat tissue
260 than in muscle. The diameter of adipocytes was larger in the high-IMF than in the low-

IMF line in both Semimembranosus proprius (P_0 = 0.99) and perirenal fat (P_0 = 0.97). In contrast, we did not find differences between lines in the number of adipocytes.

DISCUSSION

Direct response to selection represented around 1.7 SD of the trait, 24.5% of the mean, and a genetic progress of around 5% of the mean per generation. Other selection experiments for IMF in pigs (Schwab et al. 2009), chickens (Zhao et al. 2009) and cattle (Sapp et al. 2002) also obtained great direct responses to selection. Selection for IMF showed a positive correlated response in perirenal fat weight at 13 wk, but we did not find differences at 9 wk. This result could be a sampling effect; when considering the animals of the whole generation (*n* = 202), perirenal fat weight was 2.39 g greater in high-IMF line compared to low-IMF line (P_0 = 1.00), with 95% confidence interval [1.25, 3.47]. A positive correlated response to selection for IMF in carcass fat has been observed in pigs (Schwab et al. 2009) and chickens (Zhao et al. 2007), but not in the selection experiment in cattle (Sapp et al. 2002), where selection was performed only during one generation.

The difference between lines for IMF at 9 wk in Longissimus dorsi was 0.26 g of IMF/100 g muscle. Besides, selection for IMF showed a positive correlated response in the IMF of Semimembranosus proprius at 9 wk of age (Martínez-Álvaro et al. 2015 in a study from this selection experiment). However, differences between lines in IMF at 9 wk in both muscles with different oxidative pattern were apparently not explained by differences in lipogenic activities in muscle tissue. The lack of differences between lines found in several enzymatic activities should be taken with caution because of large $HPD_{95\%}$. Gondret et al. (1997) pointed out that intramuscular adipose site displays a slower rate of development compared to other adipose tissue in rabbits. Differences in

IMF at 9 wk of age could be related to lipogenic activity in liver, which is the major lipogenic tissue in growing rabbits (Leung and Bauman, 1975 and Véizhnet and Nougues, 1977).

At 13 wk of age the IMF differences between lines are greater than at 9 wk in Longissimus dorsi and Semimembranosus proprius (Martínez-Álvaro et al. 2015), which is in line with the differences observed in lipogenic activities. Differences expressed in units of SD allow studying their relevance. Lines especially differed in the G6PDH activity in Longissimus dorsi. In this muscle, differences between lines were 1.13 SD for G6PDH, 0.47 SD for EM and 0.54 SD for FAS. Enzyme G6PDH is involved in the pentose phosphate pathway and produces NADPH required for de novo synthesis of fatty acids. This enzyme has been related to marbling in cattle (Bonnet et al. 2007). In Semimembranosus proprius, G6PDH and FAS activities were affected by selection in a similar way (differences between lines at 13 wk were 0.62 SD for G6PDH and FAS activities).

In perirenal fat, differences between lines at 13 wk were 0.43 SD for G6PDH, 0.41 SD for EM and 0.67 SD for FAS. In this tissue, the greater G6PDH and FAS activities in the high-IMF line are according to its greater perirenal fat weight. Although EM activity was greater in the low-IMF than in the high-IMF line, perirenal fat shows greater G6PDH than EM activity, indicating that G6PDH is the main supplier of NADPH in this tissue. At 9 wk, we did not find differences between lines in perirenal fat weight in this sample, which can explain the lack of differences observed in their lipogenic activities.

This is the first work that studies the lipogenic activities of muscles and fat depots in animals divergently selected for IMF. Steele et al. 1972 studied the effect of

310 divergent selection for backfat thickness in pigs and found greater EM and G6PDH
311 activities in the subcutaneous fat tissue of the fat line. Several studies have related
312 greater lipogenic activities to greater IMF in rabbits (Zomeño et al. 2010), pigs
313 (Ramírez et al. 2007 and Mourot and Kouba 1998 and 1999) and cattle (Bonnet et al.
314 2007 and Ward et al. 2010). Besides, greater lipogenic activities have been also related
315 to greater carcass fat depots in pigs (Mourot et al. 1996, Ramírez et al. 2007 and Hood
316 and Allen et al. 1973) and cattle (Bonnet et al. 2007). However, all these studies
317 compared breeds with different genetic backgrounds, whereas in our study animals
318 shared the same genetic origin, only differing in IMF and correlated traits.

319 In our experiment, catabolic activities do not apparently explain the differences
320 between lines in IMF in any muscle. In other selection experiments for IMF, catabolic
321 activities of muscles were not measured. In several species, greater fatty acid oxidation
322 has been related to lower IMF (Zomeño et al. 2010 in rabbits, Young et al. 2002 in rats,
323 Kim et al. 2000 in humans, Gondret and Lebret, 2007 in pigs and Hocquette et al. 2012
324 in cattle). The LDH enzyme is involved in the ATP production from glucose in muscle.
325 Previous studies in rabbits (Zomeño et al. 2010) and cattle (Hocquette et al. 2012) did
326 not find any relationship between LDH activity and IMF. On the other side, lipolytic
327 enzymes in muscle degrade IMF releasing free fatty acids for metabolism requirements
328 (Zechner et al. 2012). However, the relationship between lipolytic activity in muscle
329 and IMF is not clear. Some studies have related greater IMF deposition to greater
330 lipolytic activities (Hernández et al. 2008 in rabbits and Cánovas et al. 2010 in pigs),
331 while other studies have related it to lower lipolytic activities (Zomeño et al. 2010 in
332 rabbits, Cava et al. 2004 in pigs and Jeong et al. 2012 in cattle). In two lines of pigs
333 divergently selected for backfat thickness, lipolysis could not explain the different fat
334 deposition between lines (Mersmann et al. 1985). However, all these studies are

developed with animals from different genetic origins, selected for different criterions or subjected to different feeding treatments, but none of them have evaluated animals that differ exclusively in IMF and correlated traits. Thus, comparisons with our results should be taken with caution.

The greater fat deposition of the high-IMF line can be ascribed to larger adipocytes in Semimembranosus proprius and perirenal fat tissues respect to the low-IMF line. We did not observe differences between lines in the number of adipocytes; however, this trait showed a great variation, in comparison with adipocytes diameter, and differences were estimated with low accuracy (large $HPD_{95\%}$). Previous selection experiments for IMF did not study the adipocytes characteristics of their lines. In the divergent selection experiment of backfat thickness in pigs, the fat line had greater size and number of adipocytes than the lean line in fat depots in animals slaughtered at 100 days (Steele et al. 1974), although adipocyte size was concluded to be more related to total carcass fat (Steele et al. 1973). Other studies in pigs ascribed IMF variations to the size (Hauser et al., 1997) and to the number of adipocytes (Damon et al. 2006).

In summary, differences between lines for IMF after genetic selection were explained by differences in the lipogenic activities in muscles with diverse oxidative pattern at 13 wk, but not at the selection age (9 wk). Particularly, lines showed a great difference in the G6PDH activity of Longissimus dorsi muscle at 13 wk. In contrast, the different IMF deposition of the lines was apparently not explained by different catabolic activities in muscles. In perirenal fat weight, differences between lines at 13 wk correspond to differences in G6PDH and FAS activities in this tissue. The greater IMF and carcass fat of the high-IMF respect to the low-IMF line seems to be the result of larger adipocytes, whereas we did not find differences between lines in the number of adipocytes.

LITERATURE CITED

Bonnet, M., Y. Faulconnier, C. Leroux, C. Jurie, I. Cassar-Malek, D. Bauchart, P. Boulesteix, D. Pethick, J. F. Hocquette, and Y. Chilliard. 2007. Glucose-6-phosphate dehydrogenase and leptin are related to marbling differences among Limousin and Angus or Japanese Black x Angus steers. J. Anim. Sci. 85:2882–2894.

Bass, A., D. Brdiczka, P. Eyer, S. Hofer, and D. Pette. 1969. Metabolic differentiation of distinct muscle types at the level of enzymatic organization. Eur. J. Biochem. 10:198–206. Available from: http://doi.wiley.com/10.1111/j.1432-1033.1969.tb00674.x

Bergmeyer, H. U., and E. Bernt. 1974. Lactate dehydrogenase: UV-assay with pyruvate and NADH. In: N. Academic Press, New York, editor. Methods of Enzymatic Analysis. p. 574–579.

Blasco, A. and J. Ouhayoun. 1996. Harmonization of Criteria and Terminology in Rabbit Meat Research. Revised Proposal. World Rabbit Sci. 4:93–99.

Blasco, A. 2001. The Bayesian controversy in animal breeding. J. Anim. Sci. 79:2023-2046.

Blasco, A. 2005. The use of Bayesian statistics in meat quality analyses: A review. Meat Sci. 69:115-122.

Cánovas, A., R. Quintanilla, M. Amills, and R. N. Pena. 2010. Muscle transcriptomic profiles in pigs with divergent phenotypes for fatness traits. BMC Genomics 11:372. Available from: http://www.scopus.com/inward/record.url?eid=2-s2.0-77953308695&partnerID=tZOtx3y1

Cava, R., Ferrer, J.M., Estévez, M., Morcuende, D. and Toldrá, F. 2004. Composition and proteolytic and lipolytic enzyme activities in muscle Longissimus dorsi from Iberian pigs and industrial genotype pigs. Food Chemistry 88: 25-33.

Chang, H.-C., I. Seidman, G. Teebor, and M. D. Lane. 1967. Liver acetyl CoA carboxylase and fatty acid synthetase: Relative activities in the normal state and in hereditary obesity. Biochem. Biophys. Res. Commun. 28:682–686.

Damon, M., Loveau, I., Lefaucheur, L., Lebret, B., Vincent, A., Leroy, P., Sanchez, M.P., Herpin, P. and Gondret, F. 2006. Number of intramuscular adipocytes and fatty acid binding protein-4 content are significant indicators of intramuscular fat level in crossbred Large White x Duroc pigs. Journal of Animal Science 2006.84: 1083-1092.

European Economic Community. 1998. Council Directive 98/58/EC of 20 July 1998 concerning the protection of animals kept for farming purposes. Official Journal L, 221(8), 8.

European Commission Directive. 2010. Council, E. P. A. E. 2010/63/EU on the protection of animals used for scientific purposes. Institute for Health and Consumer Protection, Ispra, Italy, b7.

Fitch, W. M., R. Hill, and I. L. Chaikoff. 1959. The effect of fructose feeding on glycolytic enzyme activities of the normal rat liver. J. Biol. Chem. 234:1048–1051.

Gondret, F., Mourot, J. and Bonneau, M. 1997. Developmental changes in lipogenic enzymes in muscle compared to liver and extramuscular adipose tissues in the rabbit (Oryctolagus cuniculus). Comp. Bioch. Physiol. B Biochem. Mol. Biol. 117: 259-265.

Gondret, F. and Lebret, B. 2007. Does feed restriction and re-alimentation differently affect lipid content and metabolism according to muscle type in ìgs (Sus scrofa)?. Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology,147 82): 375-382.

Hauser, N., J. Mourot, L. De Clercq, C. Genart, and C. Remacle. 1997. The cellularity of developing adipose tissues in Pietrain and Meishan pigs. Reprod. Nutr. Dev. 37:617–625.

Hernández, P., J. L. Navarro, and F. Toldrá. 1999. Effect of frozen storage on lipids and lipolytic activities in the Longissimus dorsi muscle of the pig. Zeitschrift Leb. und - forsch. A 208:110–115.

Hernández, P., V. Cesari, and a. Blasco. 2008. Effect of genetic rabbit lines on lipid content, lipolytic activities and fatty acid composition of hind leg meat and perirenal fat. Meat Sci. 78:485–491.

Hocquette, J.-F., F. Gondret, E. Baéza, F. Médale, C. Jurie, and D. W. Pethick. 2010. Intramuscular fat content in meat-producing animals: development, genetic and nutritional control, and identification of putative markers. Animal 4:303.

Hocquette, J.F., Cassar-Malek, I., Jurie, C., Bauchart, D., Picard B. and Renand, G. 2012. Relationships between muscle growth potential, intramuscular fat content and different indicators of muscle fibre types in young Charolais bulls. Animal Science Journal 83, 750-758.

Hood, R.L. and Allen, C.E. 1973 Lipogenic anzyme activity in adiposse tissue during the growth of swine with different propensities to fatten. The Journal of nutrition, 103 (3) p. 353-362

Hsu, R. Y., and H. A. Lardy. 1969. Malic enzyme. In: N. Y. N. Academic Press, editor. Methods in enzymology. p. 230–235.

Jeong, J. Kwon, E.G., Im, S.K., Seo, K.S. and Baik, M. Expression of fat deposition and fat removal genes is associated with intramuscular fat content in Longissimus dorsi muscle of Korean cattle steers. J. Anim. Sci. 90: 2044-2053.

Kim, J.Y., Hickner, R. C., Cortright, R. L., Dohm, G.L. and Houmard, J. 2000. Lipid oxidation is reduced in obese human skeletal muscle. American journal of physiology. Endocrinology and metabolism.279 E1039-E1044.

Leung, T.T. and Bauman, D.E. 1975 In vivo studies of the site of fatty acid synthesis in the rabbit. Int. J. Biochem, 6: 801-805.

Martínez-Álvaro, M., V. Juste, A. Blasco, and P. Hernández. 2015. Response to selection for intramuscular fat content and correlated responses in several muscles in rabbits. Proceedings of the 66th Annual Meeting of the European Association of Animal Production. Warsaw (Poland), 31 August- 4 September.

Martínez-Álvaro, M., V. Juste, A. Blasco, and P. Hernández. 2016. Divergent selection for intramuscular fat in rabbits: responses to selection and genetic parameters. Journal of Animal Science, 94. doi: 10.252/jas2016-0590.

442 Mersmann, H.J. 1985. Adipose tissue lipolytic rate in genetically obese and lean swine.
443 J. Anim. Sci.60

444 Mourot, J., M. Kouba, and M. Bonneau. 1996. Comparative study of in vitro lipogenesis
445 in various adipose tissues in the growing meishan pig: Comparison with the large white
446 pig (Sus domesticus). Comp. Biochem. Physiol. Part B Biochem. Mol. Biol. 115:383–
447 388.

448 Mourot, J. and Kouba, M. 1998. Lipogenic enzyme activities in muscle of growing
449 Large White and Meishang pigs. Livestock Production Science 55: 127-133

450 Mourot, J. and Kouba, M. 1999. Development of intra- and intermuscular adipose tissue
451 in growing Large White and Meishang pigs. Reprod. Nutr. Dev. 39: 125-132.

452 Ramírez, M. R., D. Morcuende, and R. Cava. 2007. Fatty acid composition and
453 adipogenic enzyme activity of muscle and adipose tissue, as affected by Iberian×Duroc
454 pig genotype. Food Chem. 104:500–509.

455 Rodbell, M. 1964. Metabolism of isolated fat cells: I.Effects of hormones on glucose
456 metabolism and lipolysis. J. Biol. Chem. 239:375–80.

457 Sapp, R. L., J. K. Bertrand, T. D. Pringle, and D. E. Wilson. 2002. Effects of selection
458 for ultrasound intramuscular fat percentage in Angus bulls on carcass traits of progeny.
459 J. Anim. Sci. 80:2017–2022.

460 Schwab, C. R., T. J. Baas, K. J. Stalder, and D. Nettleton. 2009. Results from six
461 generations of selection for intramuscular fat in Duroc swine using real-time ultrasound.
462 I. Direct and correlated phenotypic responses to selection. J. Anim. Sci. 87:2774–2780.

463 Smith, P. K., R. I. Krohn, G. T. Hermanson, A. K. Mallia, F. H. Gartner, M. D.
464 Provenzano, E. K. Fujimoto, N. M. Goeke, B. J. Olson, and D. C. Klenk. 1985.
465 Measurement of protein using bicinchoninic acid. Anal. Biochem. 150:76–85.

466 Sorensen, D., and D. Gianola. 2002. Likelihood, Bayesian, and MCMC methods in
467 quantitative genetics. Springer, New York, NY.

468 Srere, P. A. 1969. Citrate synthase. In: N. Academic Press, New York, editor. Methods
469 in Enzymology. Vol. 13. Academic Press. p. 3–11

470 Steele, N.C., Frobish, L.T., Davey, R.J. and Keeney, M. 1972. Effect of selection for
471 backfat thickness in swine on lipogenic enzyme levels. Journal of Animal Science.
472 1972. 35 p.225.

473 Steele, N.C., Frobish, L.T. and Keeney, M. 1973. Adiposity and metabolic activity in
474 adipose tissue of swine selected for backfat thickness: age constant. Journal of Animal
475 Science. 1973. 37 (1) p 367-367.

476 Steele, N.C., Frobish, L.T. and Keeney, M. 1974. Lipogenesis and cellularity of adipose
477 tissue from genetically lean and obese swine. J. Anim. Sci 39(4):712-719.

478 Veizhnet, A. and Nouguès, J. 1977. Evolution postnatale de la lipogenèse dans le tissu
479 adipeux et le foie du mouton et du lapin. Ann. Biol. Anim. Bioch. Biophys., 5B:851-
480 863.

481 Ward, R., Woodward, B., Otter, N. and Doran, O. Relationship between the expression
482 of key lipogenic enzymes, fatty acid composition, and intramuscular fat content of
483 Limousin and Aberdeen Angus cattle. Livestock Science 127 :22-29.

484 Wood, J.D., Enser, M., Fisher, A.V., Nute, G.R., Sheard, P.R., Richardson, R.I.,
485 Hughes, S.I. and Whittington, F.M. 2008. Fat deposition, fatty acid composition and
486 meat quality: a review. Meat Science. 78:343-358.

487 Young, M.E., Guthrie, Patrick, H. Razeghi, P., Leighton, B., Abbasi, S., Patil, S.,
488 Youker, K. A. and Teagtmeyer, H. 2002 Impaired long-chain fatty acid oxidation and
489 contractile dysfunction in the obese zucker rat heart. Diabetes, 51 2587-2595.

490 Zechner, R., Zimmermann, R., Eichmann, T.O., Kohlwein, S.D., Haemmerle, G., Lass,
491 A. and Madeo, F. Fat signals- Lipases and lipolysis in lipid metabolism and signaling.
492 Cell metabolism, 15:279-291.

493 Zhao, G. P., J. L. Chen, M. Q. Zheng, J. Wen, and Y. Zhang. 2007. Correlated
494 responses to selection for increased intramuscular fat in a Chinese quality chicken line.
495 Poult. Sci. 86:2309–2314.

496 Zomeño, C., A. Blasco, and P. Hernández. 2010. Influence of genetic line on lipid
497 metabolism traits of rabbit muscle. J. Anim. Sci. 88:3419–3427.

498 Zomeño, C., P. Hernández, and A. Blasco. 2011. Use of near infrared spectroscopy for
499 intramuscular fat selection in rabbits. World Rabbit Sci. 19:203–208.

500 Zomeño, C., V. Juste and P. Hernández. 2012. Application of NIRS for predicting fatty
501 acids in intramuscular fat of rabbit. Meat Sci. 91(2):155-159

TABLES

Table 1. Descriptive statistics and differences between lines in carcass traits (g) at 9 and 13 wk of age.

	9 wk					13 wk				
Trait	Mean	SD	[1]D	[2]HPD$_{95\%}$		[3]P_0	Mean	SD	[1]D	[2]HPD$_{95\%}$		[3]P_0
Hot carcass weight	1022	90.5	-10.1	-74.9,	53.6	0.62	1645	137	-43.6	-110,	17.9	0.91
Longissismus dorsi weight	84.7	10.2	1.85	-6.59,	10.5	0.67	166	15.7	-4.07	-12.7,	5.53	0.81
Semimembranosus proprius weight	2.06	0.27	0.00	-0.21,	0.24	0.50	3.72	0.47	-0.13	-0.35,	0.09	0.88
Perirenal fat weight	7.91	3.18	0.80	-3.22,	4.01	0.67	23.3	8.57	6.62	2.96,	10.2	1.00

[1]D, median of the marginal posterior distribution of the difference between high-intramuscular fat (IMF) and low-IMF lines; [2]HPD $_{95\%}$, highest posterior density region at 95% of probability; [3]P_0, probability of the difference being greater than zero when D >0 or lower than zero when D <0.

512 Table 2. Descriptive statistics and differences between lines in lipogenic[1] activities measured in several tissues at 9 and 13 wk of age.

		9 wk						13 wk					
Tissue	Trait	Mean	SD	[2]D	[3]HPD$_{95\%}$		[4]P$_0$	Mean	SD	[2]D	[3]HPD$_{95\%}$		[4]P$_0$
Longissimus dorsi muscle	G6PDH	119	32.0	-5.81	-24.5,	10.6	0.74	130	29.7	33.4	12.1,	52.8	1.00
	EM	484	167	-42.3	-170,	83.3	0.76	740	205	96.8	-39.0,	219	0.93
	FAS	12.7	5.66	-1.26	-6.53,	4.61	0.68	16.9	6.60	3.56	-2.53,	8.77	0.90
Semimembranosus proprius muscle	G6PDH	287	84.4	-37.8	-105,	26.7	0.88	425	123	76.6	2.62,	153	0.98
	EM	378	87.6	-58.6	-118,	-0.03	0.98	414	99.4	28.3	-35.1,	89.3	0.82
	FAS	63.2	18.4	8.12	-8.02,	23.9	0.85	87.9	25.5	15.7	-2.49,	33.0	0.95
Perirenal fat depot	G6PDH	765	224	35.1	-132,	217	0.66	940	322	138	-70.3,	336	0.91
	EM	175	67.6	-9.98	-58.5,	35.1	0.67	251	83.7	-34.1	-84.7,	18.6	0.90
	FAS	265	72.8	-14.6	-79.2,	64.0	0.65	314	85.0	56.4	-6.25,	117	0.96

513

514 [1]Activities of the lipogenic enzymes glucose-6-phosphate dehydrogenase (G6PDH), malic enzyme (EM) and fatty acid synthase (FAS) in
515 nmol/min and g of tissue; [2]D, median of the marginal posterior distribution of the difference between high-intramuscular fat (IMF) and low- IMF
516 lines; [3]HPD $_{95\%}$, highest posterior density region at 95% of probability; [4]P$_0$, probability of the difference being greater than zero when D >0 or
517 lower than zero when D <0.

518

519

520 Table 3. Descriptive statistics and differences between lines in catabolic[1] activities measured in muscles at 9 and 13 wk of age.

		9 wk					13 wk				
Tissue	Trait	Mean	SD	^{2}D	^{3}HPD$_{95\%}$		Mean	SD	^{2}D	^{3}HPD$_{95\%}$		^{4}P$_0$
Longissimus dorsi muscle	HAD	1.47	0.34	0.25	-0.05,	0.53	0.96	1.43	0.38	0.13	-0.14,	0.39	0.83
	CS	3.46	0.67	0.44	-0.29,	1.25	0.88	3.39	0.85	-0.13	-0.83,	0.49	0.66
	LDH	857	182	40.7	-122,	194	0.69	1139	255	-38.0	-192,	120	0.70
	AL	0.65	0.10	0.01	-0.06,	0.07	0.58	0.54	0.08	-0.01	-0.07,	0.06	0.57
	NL	4.04	0.87	0.22	-0.28,	0.68	0.81	3.18	0.58	0.13	-0.33,	0.65	0.71
	APL	0.38	0.07	0.01	-0.03,	0.06	0.72	0.31	0.07	0.00	-0.05,	0.04	0.57
Semimembranosus proprius muscle	HAD	2.80	0.63	0.28	-0.21,	0.82	0.86	2.65	0.59	-0.15	-0.64,	0.31	0.72
	CS	5.43	1.05	0.14	-0.58,	0.85	0.65	5.10	0.98	-0.48	-1.17,	0.27	0.90
	LDH	47.7	13.2	0.30	-9.01,	8.49	0.52	49.9	13.1	1.06	-7.46,	9.95	0.60

521

522 [1]Activities of the enzymes β-hydroxyacyl-CoA dehydrogenase (HAD), citrate synthase (CS) and lactate dehydrogenase (LDH) in µmol/min and
523 g of tissue. Activities of the enzymes acid lipase (AL), neutral lipase (NL) and acid phospholipase (APL) in µmol/h and g of tissue; ^{2}D, median of
524 the marginal posterior distribution of the difference between high-intramuscular fat (IMF) and low-IMF lines; ^{3}HPD $_{95\%}$, highest posterior
525 density region at 95% of probability; ^{4}P$_0$, probability of the difference being greater than zero when D >0 or lower than zero when D <0.

Table 4. Descriptive statistics and differences between lines in adipocytes characteristics in Semimembranosus proprius muscle and perirenal fat depot.

Tissue	Trait	Mean	SD	D[1]	$HPD_{95\%}$[2]		P_0[3]
Semimembranosus proprius muscle	Adipocyte diameter, µm	35.8	3.83	4.15	0.67,	7.77	0.99
	Total nº adipocytes ($x10^6$)	4.24	1.58	-0.68	-2.46,	1.00	0.80
Perirenal fat depot	Adipocyte diameter, µm	96.5	13.4	11.0	-0.37,	23.3	0.97
	Total nº adipocytes ($x10^6$)	29.3	12.2	-2.35	-14.7,	8.50	0.67

[1]D, median of the marginal posterior distribution of the difference between high-intramuscular fat (IMF) and low-IMF lines; [2]$HPD_{95\%}$, highest posterior density region at 95% of probability; [3]P_0, probability of the difference between lines being greater than zero when D >0 or lower than zero when D <0.