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

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ABSTRACT: A divergent selection experiment for intramuscular fat (IMF) of LM at 9 wk of age was performed in rabbits. The objective of this work was 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 2 muscles with different oxidative patterns (LM and semimembranosus proprius) and in the perirenal fat depot at 2 ages, 9 and 13 wk. In addition, adipocytes were characterized in perirenal fat. In the fifth generation, direct response to selection was 0.26 g IMF/100 g muscle. Lines showed differences in their lipogenic activities of muscles and fat tissues at 13 wk but not at 9 wk. The high-IMF line showed greater glucose-6-phosphate dehydrogenase (G6PDH), malic enzyme (EM), and fatty acid synthase (FAS) activities in LM than the low-IMF line, with probabilities = 1.00, 0.93, and 0.90, respectively. Differences between lines were particularly great for G6PDH activity, representing 1.13 SD. The high-IMF line also showed greater G6PDH and FAS activities in semimembranosus proprius ($P_0 = 0.98$ for G6PDH and 0.95 for FAS) and perirenal fat ($P_0 = 0.91$ for G6PDH and 0.96 for FAS). However, in perirenal fat, EM activity was greater in the low-IMF line ($P_0 = 0.90$). No differences between lines were found in almost any catabolic or lipolytic activities of muscles. Regarding adipocyte characteristics, the high-IMF line showed larger adipocytes in perirenal fat depot tissue ($P_0 = 0.97$) compared to the low-IMF line, but no differences between lines were observed in the number of adipocytes. This study sheds light on the metabolic activities involved in the genetic differentiation of lipid deposition in rabbits. This study shows that lipogenic activities in muscles and fat tissues, in particular G6PDH in LM, are involved in the lipid accumulation in muscle and adipose tissues.

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 because of its adequate variability and high heritability (see Ciobanu et al., 2011, for pigs; Mateescu, 2015, for cattle; Martínez-Álvaro et al., 2016, for rabbits). However, only 3 selection experiments for IMF have been published (Sapp et al., 2002, for cattle; Zhao et al., 2007, for chickens; Schwab et al., 2009, for pigs). At the Universitat Politècnica de València we are performing a divergent selection experiment for IMF of LM in rabbits (Zomeño et al., 2013; Martínez-Álvaro et al., 2016). Generally, commercial carcasses of rabbits vary between countries from 9 to 13 wk of age, showing greater IMF content at the older age.

An increased IMF content has been related to greater lipogenic rate in muscle and changes in catabolic activities in several species, including rabbits (Hernández et al., 2008; Zomeño et al., 2010), pigs (Mourot and Kouba, 1998, 1999; Gondret and Lebret, 2007), and cattle (Bonnet et al., 2007; Hocquette et al., 2012). In addition, differences in the amount of

fat have been ascribed to differences in the size and/or number of adipocytes (Steele et al., 1973, 1974, 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 (such as perirenal fat weight or IMF in several muscles, reported in Martínez-Álvaro et al., 2015, 2016). None of the previous selection experiments for IMF analyzed the lipid metabolism of their lines.

The longissimus dorsi is a predominantly white fast-twitch muscle. Selection for IMF on this muscle could affect in different ways the lipid metabolism of muscles having other oxidative patterns (such as the pure oxidative semimembranosus proprius muscle) and adipose tissues. Along these lines, Quintanilla et al. (2011) suggested that IMF content is regulated by QTL with different muscle-specific effects. The objective of this work is to compare the lipid metabolism and adipocytes cellularity of muscles with diverse oxidative patterns and fat tissues at 9 and 13 wk of age in 2 rabbit lines divergently selected for IMF.

MATERIALS AND METHODS

Animals

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 Parliament and the Council of the European Union, 2010).

This study was performed with rabbits from the fifth generation of a divergent selection experiment for IMF of the LM at 9 wk of age. Animals came from a synthetic rabbit line. The base population consisted of 13 males and 83 females. Lines selected for high and low IMF had 8 males and 40 females per generation. Intramuscular fat content was evaluated in 2 rabbits (a male and a female) from the first parity of each doe, and the average between these 2 phenotypic 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 the next generation. Each sire was mated with 5 does, and to reduce inbreeding, only 1 male progeny of the sire, from the highest ranked mate, was selected for breeding the next generation. 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 was used to select the rabbits for the next generation, although some exceptions were made, resulting in some IMF measurement collection during the second or third parity. More details of this experiment can be found in Zomeño et al. (2013) and Martínez-Álvaro et al. (2016). Litters were homogenized 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. The housing had a constant photoperiod of 16:8 h and controlled ventilation.

In the fifth generation, direct response to selection was estimated using 202 rabbits (100 from the high-IMF line and 102 from the low-IMF line). Between them, 160 came from the first parity, 24 from the second parity, and 18 from the third parity. Rabbits were slaughtered at 9 wk using electrical stunning and exsanguination. After slaughter, carcasses were chilled for 24h at 4°C. Then, the LM was excised, minced, freeze-dried, and scanned with near-infrared spectrophotometry (model 5000, FOSS NIRSystems Inc., Hilleroed, Denmark) to measure IMF using the prediction equations developed by Zomeño et al. (2011, 2012). Intramuscular fat was expressed as grams per 100 grams of muscle on a fresh basis.

Enzyme activities were measured in an additional sample of 110 rabbits, 62 slaughtered at 9 wk (32 from the high-IMF line and 30 from the low-IMF line) and 48 slaughtered at 13 wk (24 per line). All of these rabbits came from the first parity. Adipocytes were characterized in another additional sample of 45 rabbits, 23 slaughtered at 9 wk (10 from the high-IMF line and 13 from the low-IMF line) and 22 slaughtered at 13 wk (10 from the high-IMF line and 12 from the low-IMF line), all of them from the third parity. Animals used for enzymatic assays and adipocytes measurements were slaughtered as described above. Immediately after slaughter, HCW was registered according to the norms proposed by the World Rabbit Science Association (Blasco and Ouhayoun, 1996), and LM and semimembranosus proprius muscle and perirenal fat depot were excised and weighed. For enzymatic assays, samples of the 3 tissues were frozen in liquid nitrogen and stored at −80°C. For adipocytes measurements, samples of 0.5 g of perirenal fat were collected immediately after slaughter and placed in tubes with 10 mL of Tyrode's solution with a pH of 7.6 (0.15 M NaCl, 6 mM KCl, 2mM CaCl$_2$, 6 mM glucose, 2 mM NaHCO$_3$) at 39°C.

Measurement of Enzyme Activities

Activities of the lipogenic enzyme glucose-6-phosphate dehydrogenase (**G6PDH**), malic enzyme (**EM**), and fatty acid synthase (**FAS**) were measured in LM and semimembranosus proprius muscle and perirenal fat. A quantity of tissue (1 g for LM 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 1 mM EDTA (2.5 mL for muscles and 5 mL for perirenal fat). Homogenates were centrifuged at 12,000 × g for 1 h at 4°C, and supernatants were

filtered through glass wool and collected for enzyme assays. Activities were assessed at 37°C 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 nanomoles of nicotinamide adenine dinucleotide phosphate (**NADPH**) produced for G6PDH and EM or oxidized for FAS per minute and grams of fresh tissue.

Activities of catabolic enzymes β-hydroxyacyl-CoA dehydrogenase (**HAD**), citrate synthase (**CS**), and lactate dehydrogenase (**LDH**) were determined in LM and semimembranosus proprius muscle. Samples of 0.2 g of muscle tissue were homogenized in 50 wt/vol of ice cold 0.1 M phosphate buffer (pH 7.5) and 2 mM EDTA. Homogenates were centrifuged at 6,000 × g for 15 min at 4°C, and the resulting supernatants were filtered as described above. Enzymatic activities were assessed at 30°C in a spectrophotometric analyzer (Fluostar Galaxy, BMG Lab Technologies, Offenburg, Germany) at 340 nm for HAD and LDH and 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 micromoles of NADH for HAD and LDH or of mercaptide ion for CS released per minute and gram of fresh tissue.

Lipolytic enzyme activities of acid lipase (**AL**), neutral lipase (**NL**), and acid phospholipase (**APL**) were assayed on the LM, according to the method described by Hernández et al. (1999) using 4-methylumbelliferyloleate as the fluorescent substrate. Lipolytic activity was measured only in LM because of the lack of semimembranosus proprius sample remaining after other analyses. A sample of 4 g of LM 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 10,000 × g for 20 min at 4°C, and the resulting supernatant was filtered as described above. Reaction mixtures of lipase assays with fluorometric substrates were incubated at 37°C for 20 min. The fluorescence was measured at an excitation wavelength of 460 nm using a Fluostar Galaxy fluorometer (BMG Lab Technologies). Enzyme activities were expressed as micromoles of substrate hydrolyzed per hour and gram of fresh tissue.

Soluble protein was determined from supernatant in LM and semimembranosus proprius muscle using the bicinchoninic acid protein assay kit (Smith et al., 1985) provided by Pierce (Rockford, IL), and enzyme activities were also expressed in a soluble-protein content basis.

Characterization of Adipocytes

Perirenal fat samples underwent digestion with collagenase (Rodbell, 1964). Approximately 100 mg of

each sample were digested with 0.1 mg of collagenase type 2, 4 mg of bovine serum albumin, and 100 μL of T199 medium at 39°C for 1 h. 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, MD). Adipocytes diameter was determined from 300 cells, and the average diameter was calculated per sample. Adipocytes with diameters less than 20 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 grams per 100 g of fresh tissue. The number of adipocytes per gram of tissue was calculated by dividing the lipid content in a gram of sample by the lipid content of 1 adipocyte (assuming a lipid density value of 0.915 g/mL and cells to be spherical in shape). The total number of adipocytes was calculated by multiplying the number of adipocytes per gram of tissue by the weight of the tissue.

Statistical Analysis

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 for the selection trait were collected during a long period of time, the effect of month-season was included in the analysis. For enzymatic activities and adipocyte characteristics the effect of parity order was not included in the analysis since animals came from the same parity. For adipocyte characteristics and lipid content, records at 9 and 13 wk were analyzed together, correcting data by the effect of age.

The 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 lipogenic activities in perirenal fat tissue we repeated the analysis including perirenal fat weight as a covariate. For adipocyte characteristics and lipid content, differences between lines were estimated with all data, including the additional fixed effect of age. We also estimated the differences between ages for enzymatic activities using all data and including the 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 (Blasco, 2001, 2005, 2017). 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

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	D[1]	HPD$_{95\%}$[2]	P$_0$[3]	Mean	SD	D[1]	HPD$_{95\%}$[2]	P$_0$[3]
HCW	1,022	90.5	−10.1	−74.9, 53.6	0.62	1,645	137	−43.6	−110, 17.9	0.91
LM 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.

time series procedures (Sorensen and Gianola, 2002). Chains of 60,000 samples with a burn-in of 10,000 were used. The program 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 were the median (**D**), the highest posterior density region at 95% (**HPD$_{95\%}$**), and the probability of the lines being different, calculated as the probability of the line difference being greater than zero given the absolute value of D (**P$_0$**). More details of these Bayesian parameters 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 the LM at 9 wk, was 1.03 g IMF/100 g muscle with a SD of 0.15. The direct response to selection estimated as the differences between high- and low-IMF lines was 0.26 g IMF/100 g muscle (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 represented a low percentage of the HCW (0.77% at 9 wk and 1.41% at 13 wk). Perirenal fat weight was greater in the high-IMF line than in the low-IMF line at 13 wk of age (P$_0$ = 1.00), but no difference was observed between lines at 9 wk. The difference between lines at 13 wk represented 0.43% of the HCW. No differences between lines were observed in HCW and muscle weights at any age, except for HCW at 13 wk, which was greater in the low-IMF line than in the high-IMF line (P$_0$ = 0.91).

Lipogenic Enzyme Activities in Muscles and Perirenal Fat

Table 2 shows descriptive statistics and differences between lines in lipogenic activities in muscles and perirenal fat at 9 and 13 wk in nanomoles per minute per gram of tissue. The semimembranosus proprius muscle showed greater G6PDH and FAS activities and lower EM activity compared to LM at both 9 and 13 wk. However, EM activity was greater in semimembranosus proprius when the results were expressed in a soluble-protein basis because of the low protein content of this muscle (data not shown). Perirenal fat tissue showed greater G6PDH and FAS activities and lower EM activity than the muscles at both ages.

All the lipogenic activities were greater at 13 wk than at 9 wk in the 3 tissues. The differences between 13 and 9 wk of age (in nmol·min^{-1}·g^{-1} tissue) were 11.6 for G6PDH (P$_0$ = 0.96), 265 for EM (P$_0$ = 1.00), and 4.77 for FAS (P$_0$ = 0.99) in the LM; 139 for G6PDH (P$_0$ = 1.00), 38.2 for EM (P$_0$ = 0.96), and 24.8 for FAS (P$_0$ = 1.00) in the semimembranosus proprius muscle; and 175 for G6PDH (P$_0$ = 1.00), 72.5 for EM (P$_0$ = 1.00), and 52.6 for FAS (P$_0$ = 0.99) in perirenal fat.

Lines showed differences in their lipogenic activities at 13 wk but not at 9 wk of age. At 13 wk, the high-IMF line showed greater G6PDH (P$_0$ = 1.00), EM (P$_0$ = 0.93), and FAS (P$_0$ = 0.90) activities in LM and greater G6PDH (P$_0$ = 0.98) and FAS (P$_0$ = 0.95) activities in semimembranosus proprius than the low-IMF line, whereas there was less evidence for the difference between lines in EM activity in this muscle (P$_0$ = 0.82). Similar results were obtained when lipogenic activities were expressed in a soluble-protein basis for muscles tissues (data not shown). In perirenal fat, the high-IMF line had greater G6PDH (P$_0$ = 0.91) and FAS (P$_0$ = 0.96) activities but lower EM activity than the low-IMF line (P$_0$ = 0.90). At 9 wk, we did not observe differences between lines in lipogenic activities, except for EM activity in semimembranosus proprius that was greater in the low-IMF line than in the high-IMF line (P$_0$ = 0.98). Similar results were obtained when perirenal fat weight was considered as a covariate in the model (data not shown).

Catabolic Enzyme Activities in Muscles

Table 3 shows descriptive statistics and differences between lines in oxidative and glycolytic (expressed in μmol·min^{-1}·g^{-1} tissue) and lipolytic (in μmol·h^{-1}·g^{-1}

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

Tissue	Trait	9 wk					13 wk				
		Mean	SD	D^2	$HPD_{95\%}{}^3$	$P_0{}^4$	Mean	SD	D^2	$HPD_{95\%}{}^3$	$P_0{}^4$
LM	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

[1]Activities (in nmol·min^{-1}·g^{-1} tissue) of the lipogenic enzymes glucose-6-phosphate dehydrogenase (G6PDH), malic enzyme (EM), and fatty acid synthase (FAS).

[2]D = median of the marginal posterior distribution of the difference between high–intramuscular fat (IMF) and low- IMF 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 lower than zero when D < 0.

tissue) activities in LM and semimembranosus proprius muscle at 9 and 13 wk of age. The longissimus dorsi muscle had greater LDH activity, whereas semimembranosus proprius showed greater HAD and CS activities. Similar results were obtained when activities were expressed in a soluble-protein basis (data not shown). Oxidative and glycolytic activities were similar at both ages, except for HAD activity in semimembranosus proprius, which decreased with age; the difference between ages (13 wk minus 9 wk) in HAD activity was −0.22 μmol·min^{-1}·g^{-1} tissue (P_0 = 0.93). Lipolytic activities in LM decreased with age (P_0 = 1.00); the differences between ages (13 wk minus 9 wk; in μmol·h^{-1}·g^{-1} tissue) were −0.11 for AL (P_0 = 1.00), −0.89 for NL (P_0 = 1.00), and −0.07 for APL (P_0 = 1.00).

We almost did not find differences between lines in the catabolic activities of muscles. Few differences between lines were observed in oxidative activities, although results were not consistent between muscles or ages. The high-IMF line showed greater HAD activity in LM only at 9 wk (P_0 = 0.96) compared to the low-IMF line, which showed greater CS activity in semimembranosus proprius only at 13 wk (P_0 = 0.90). We did not observe differences between lines in LDH or lipolytic activities. Results did not change when activities were expressed in a soluble-protein basis (data not shown).

Adipocyte Characteristics in Perirenal Fat

Table 4 shows descriptive statistics and differences between lines in adipocyte characteristics of perirenal fat. The diameter of adipocytes was greater in the high-IMF line than in the low-IMF line (P_0 = 0.97). In contrast, we did not find differences between lines in the number of adipocytes.

DISCUSSION

Direct response to selection represented 1.7 SD of the trait, 24.5% of the mean, and a genetic progress of approximately 5% of the mean per generation. Other selection experiments for IMF in pigs (Schwab et al., 2009), chickens (Zhao et al., 2007), 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 of age, 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 the high-IMF line compared to the low-IMF line (P_0 = 1.00), with a 95% CI of [1.25, 3.47]. A positive genetic correlation between IMF and perirenal fat was estimated with data from these selection lines (0.33; Martínez-Álvaro et al., 2016). 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 1 generation.

In the fifth generation, the difference between lines for IMF at 9 wk in LM was 0.26 g IMF/100 g muscle. Additionally, a previous study within these selection lines showed a positive correlated response in the IMF of semimembranosus proprius at 9 wk of age (Martínez-Álvaro et al., 2015). However, differences between lines in IMF at 9 wk were not explained by differences in lipogenic activities in muscle tissues with different oxidative patterns. The lack of line differences in several enzymatic activities should be taken with caution because of large $HPD_{95\%}$. Gondret et al. (1997) pointed out that i.m. adipose tissue displays a slower rate of development compared to other adipose tissues

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

Tissue	Trait	9 wk					13 wk				
		Mean	SD	D[2]	HPD$_{95\%}$[3]	P$_0$[4]	Mean	SD	D[2]	HPD$_{95\%}$[3]	P$_0$[4]
LM	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

[1]Activities of the enzymes β-hydroxyacyl-CoA dehydrogenase (HAD; μmol·min^{-1}·g^{-1} tissue), citrate synthase (CS; μmol·min^{-1}·g^{-1} tissue), and lactate dehydrogenase (LDH; μmol·min^{-1}·g^{-1} tissue) and acid lipase activity (AL; μmol·h^{-1}·g^{-1} tissue), neutral lipase activity (NL; μmol·h^{-1}·g^{-1} tissue), and acid phospholipase activity (APL; μmol·h^{-1}·g^{-1} tissue).

[2]D = median of the marginal posterior distribution of the difference between high–intramuscular fat (IMF) and low-IMF 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 lower than zero when D < 0.

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

At 13 wk of age the IMF differences between lines were greater than at 9 wk in LM and semimembranosus proprius (Martínez-Álvaro et al., 2015), which corresponds well with the line differences observed in lipogenic activities. Differences expressed in units of SD allow a comparison of their magnitude. Lines especially differed in the G6PDH activity in LM. In this muscle, differences between high- and low-IMF lines were 1.13 SD for G6PDH, 0.47 SD for EM, and 0.54 SD for FAS. The enzyme G6PDH is involved in the pentose phosphate pathway and produces NADPH required for de novo synthesis of fatty acids. Our results indicate that G6PDH activity must explain at least part of the genetic variability on IMF deposition in rabbits. This enzyme has also been related to marbling in cattle (Bonnet et al., 2007). In the semimembranosus proprius muscle, G6PDH and FAS activities were affected by selection in a similar way, with differences between lines at 13 wk of 0.62 SD for G6PDH and FAS activities.

In perirenal fat, differences between high- and low-IMF 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 were consistent with its greater perirenal fat weight at 13 wk. However, the high-IMF line showed greater G6PDH and FAS activities also when perirenal fat weight was considered as a covariate in the model. Malic enzyme activity was greater in the low-IMF than in the high-IMF line. However, perirenal fat showed greater G6PDH than EM activity, suggesting that G6PDH is

the main supplier of NADPH to this tissue in rabbits, as previously observed in cattle (Bonnet et al., 2007) and pigs (Ramírez et al., 2007). At 9 wk, we did not find differences between lines in perirenal fat weight in this sample, which could explain the lack of differences observed in their lipogenic activities.

This work is the first to study the lipogenic activities of muscles and fat depots in animals divergently selected for IMF. Steele et al. (1972) studied the effect of divergent selection for back fat thickness in pigs and found greater EM and G6PDH activities in the s.c. fat tissue of the fat line. Several studies have related greater lipogenic activities or lipogenic gene expression to greater IMF in rabbits (Zomeño et al., 2010), pigs (Mourot and Kouba, 1998, 1999; Ramírez et al., 2007), and cattle (Bonnet et al., 2007; Ward et al., 2010). In addition, greater lipogenic activities have also been related to greater carcass fat depots in pigs (Hood and Allen, 1973; Mourot et al., 1996; Ramírez et al., 2007) and cattle (Bonnet et al., 2007). However, all these studies compared breeds with different genetic backgrounds, whereas in our study animals shared the same genetic origin, differing only in IMF and correlated traits.

Longissimus dorsi and semimembranosus proprius muscles showed different oxidative patterns. In this experiment, catabolic activities did not explain the differences between lines in IMF in either muscle studied. In other selection experiments for IMF, catabolic activities of muscles were not measured. In several species, greater fatty acid oxidation has been related to lower IMF (Kim et al., 2000, in humans; Young et al., 2002, in rats; Gondret and Lebret, 2007, in pigs; Zomeño et al., 2010, in rabbits; Hocquette et al., 2012, in cattle). The LDH enzyme is involved in ATP production from glucose in muscle.

Table 4. Descriptive statistics and differences between lines in adipocyte characteristics of the perirenal fat depot

Trait	Mean	SD	D[1]	HPD$_{95\%}$[2]	P$_0$[3]
Adipocyte diameter, µm	96.5	13.4	11.0	−0.37, 23.3	0.97
Total adipocytes, × 10^6	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.

This study did not show any relationship between IMF and LDH activity. Previous studies in rabbits (Zomeño et al., 2010) and cattle (Hocquette et al., 2012) also did not find any relationship between LDH activity and IMF. On the other hand, lipolytic enzymes in muscle degrade IMF, releasing FFA for metabolism requirements (Zechner et al., 2012). However, in this study lipolytic activities were not related to IMF. The relationship between lipolytic activity in muscle and IMF is not clear. Some studies have related greater IMF deposition to greater lipolytic activities (Hernández et al., 2008, in rabbits) or greater expression of genes involved in the lipolysis (Cánovas et al., 2010, in pigs), whereas other studies have related it to lower lipolytic activities (Cava et al., 2004, in pigs; Zomeño et al., 2010, in rabbits) or lower lipolysis gene expression (Jeong et al., 2012, in cattle). In 2 lines of pigs divergently selected for back fat thickness, lipolysis could not explain the different fat deposition between lines (Mersmann, 1985). However, all these studies were developed with animals from different genetic origins, selected for different criteria or subjected to different feeding treatments, but none of them evaluated animals that differ exclusively in IMF and correlated traits. Thus, comparisons with our results should be made with care.

Because of the large variability of the adipocyte measurements and the low number of samples per each age-line group ($n = 10$), the HPD$_{95\%}$ of the differences between lines at 9 and 13 wk were very large, and we were not able to make any statement (data not shown). Analyzing the effect of selection pooling samples from both ages ($n = 20$) led to higher accurate estimations of the differences between lines. The greater carcass fat deposition of the high-IMF line can be ascribed to larger adipocytes in perirenal fat tissues with respect to the low-IMF line. Larger adipocytes can be related to the greater lipogenic activity in this tissue in the high-IMF line. A greater adipocyte volume has been closely related to greater lipogenic activities in pigs (Lee and Kauffman, 1974) and to greater lipogenic gene expression in cattle (Baik et al., 2014). We did not observe differences between lines in the number of adipocytes; however, this trait showed a great variation in comparison with adipocyte diameter, and differences were estimated with low

accuracy (i.e., large HPD$_{95\%}$). Previous selection experiments for IMF did not study the adipocyte characteristics of their lines. In the divergent selection experiment of back fat 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).

This study sheds light on the metabolic activities involved in the genetic differentiation of lipid deposition in rabbits. Differences between lines after 5 generations of selection for IMF were partially explained by differences in the lipogenic activities in muscles with a diverse oxidative pattern at 13 wk but not at the selection age (9 wk). Particularly, lines showed a greater difference in the G6PDH activity of the LM at 13 wk. In contrast, the different IMF depositions of the lines were obviously not explained by different catabolic activities in muscles. For perirenal fat weight, differences between lines at 13 wk correspond to differences in G6PDH and FAS activities in this tissue. The greater perirenal fat weight of the high-IMF line with respect to the low-IMF line appeared to be the result of larger adipocytes, whereas we did not find differences between lines in the number of adipocytes.

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