RELATIONSHIPS BETWEEN LIPID METABOLISM TRAITS IN CARCASS AND MUSCLE ADIPOSE TISSUES

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ABSTRACT

The relationships between lipid synthesis and degradation pathways were studied in order to understand the biological mechanisms involved in carcass and muscle fat deposition. A total of 120 animals from three synthetic lines (A, V and R) at two slaughter ages (9 and 13 weeks) were used. Perirenal fat adipose tissue (PF) and two muscles, *Longissimus dorsi* (LM) and *Semimembranosus proprius* (SP) were studied. Residual correlations were calculated after line and age effects were removed from the data. There was a relationship between lipogenic activity and fat content in perirenal fat tissue, r=0.37 for glucose-6-phosphate dehydrogenase (G6PDH) and r= 0.28 for malic enzyme (ME). The amount of PF has an influence on its fatty acid (FA) composition, showing positive correlations between PF content and saturated FA (SFA) (r=0.29) and monounsaturated FA (MUFA) percentage (r=0.33) and negative with polyunsaturated FA (PUFA) percentage (r=-0.55). Lipogenic enzyme activities were also related to FA composition of PF tissue, with positive correlations between lipogenic enzymes and SFA and negative with MUFA and PUFA. No association was found between IMF content of LM and its lipogenic activity. However, there was a relationship between IMF of LM and lipogenic activity of SP muscle (r>0.42). As in PF tissue, IMF content was positively related to SFA (r=0.35) and MUFA percentages (r=0.29) and negatively related to PUFA percentages (r=-0.43). In LM catabolic enzymes were also studied. There was no relationship between IMF and HAD, CS and LDH. Anabolic and catabolic pathways were related in muscle and the coordinated use of both pathways was determinant for IMF deposition. No relationship was observed among glycolytic, oxidative and lipolytic activities in LM. Moreover, the present study showed a lack of relationship between PF and IMF adipose tissues (r=-0.02). This correlation supports the idea of independence between IMF and carcass fat, suggesting the possibility of increasing intramuscular adipose tissue without increasing overall adiposity carcass.

Key words: Intramuscular fat, metabolism, perirenal fat, residual correlations.

INTRODUCTION

Intramuscular fat (IMF) content is one of the main factors affecting meat quality. Meat lipids are mainly composed by triacylglycerols (TAG). The synthesis of TAG proceeds from fatty acids (FA) synthesized *de novo* or supplied by the diet. Liver and adipose tissue are the major sites of FA synthesis in mammals. In growing rabbits, liver is the major lipogenic site, whereas adipose tissue plays an important role in adults (Gondret *et al*., 1997). Lipid deposition should be considered as the result of a balance between FA synthesis and degradation pathways.

Glucose-6-phosphate dehydrogenase (G6PDH) and malic enzyme (ME) are involved in supplying NADPH required in FA biosynthesis. Positive relationships between the activity of these enzymes and muscle lipid content were found (Gondret *et al*., 1997). Fatty acid synthase (FAS) catalyzes the last step in the FA biosynthetic pathway. Its tissue concentration is a key determinant for FA *de novo* synthesis (Clarke, 1993). The first step in lipid degradation is the hydrolysis of TAG by lipases. Fatty acids are degraded by the β-oxidation process where β-hydroxyacyl-CoA dehydrogenase (HAD) and citrate synthase (CS) are involved. Jurie *et al.* (2007) found positive correlations between

mitochondrial oxidative enzyme (HAD and CS) activities and TAG content, suggesting that FA turnover may favour TAG deposition.

The aim of this study was to examine the relationship between lipid synthesis and degradation pathways in order to understand the biological mechanisms involved in carcass and muscle fat deposition.

MATERIALS AND METHODS

Animals and experimental design

A total of 120 animals from three synthetic lines A, V and R (40 animals per line) were used in this experiment. Lines A and V were selected for litter size at weaning and line R for growth rate between weaning and slaughter (9 weeks). Animals were slaughtered by electrical stunning and exsanguination at 9 or 13 weeks of age (60 animals per age group). After slaughter, perirenal fat (PF) and two muscles, *Longissimus dorsi* (LM) and *Semimembranosus proprius* (SP), were excised from the carcass. Samples were weighed, frozen in liquid nitrogen, vacuum-packed and stored at -80 ºC until analysis.

Chemical Analyses

Activities of lipogenic enzymes G6PDH, ME and FAS were measured on LM and SP and PF tissue (n=48 per tissue). One g for LM and PF or 500 mg for SP were homogenized in 2.5 mL of 0.25*M* sucrose solution (1m*M* dithiothreitol and 1m*M* EDTA). Homogenates were centrifuged at 10000 x *g* for 1hour at 4ºC. Activities of G6PDH, ME and FAS were assessed at 37 ºC and 340 nm using a spectrophotometer (model UV-1601, Shimadzu) according to the methodology described in Zomeño *et al*. (2010). Enzyme activities were expressed in nmol of NADPH per min and per g of tissue.

The activity of enzymes HAD, CS and lactate dehydrogenase (LDH) were determined in LM and SP (n=48 per muscle). Each sample (200 mg) was homogeneized in 0.1*M* phosphate buffer (pH 7.5) (2m*M* EDTA). Homogenates were centrifuged at 6000 x *g* for 15 min at 4ºC. Enzyme activities were assayed according to the methodology described in Zomeño *et al*. (2010). Assays were conducted at 30 ºC in a Fluostar Galaxy analyzer (BMG Lab Technologies) at 340 nm (HAD and LDH) or at 405 nm (CS). Activities were expressed as mmoles of NADH (HAD, LDH) or of mercaptide ion (CS) released per min and per g of tissue.

Lipolytic enzymes acid lipase (AL), neutral lipase (NL) and acid phospholipase (APL) were assayed on LM (n=65) according to the methodology described by Hernández *et al.* (1999). Activities were expressed as mmoles of substrate hydrolyzed per h and per g of tissue.

Lipid content was determined in LM (n=85) by ether extraction (Soxtec 1043 extraction unit, Tecator) and was expressed as g per 100 g of fresh tissue.

Fatty acid methyl esters (FAME) of LM and PF were prepared as described by O´Fallon *et al*. (2007) in 65 and 48 samples, respectively. Fame were analyzed in a Focus Gas Chromatograph (Thermo) equipped with a split/splitless injector and a flame ionization detector. The separation of Fame was performed in a fused silica capillary column SPTM 2560 (Supelco). The carrier gas was Helium. The samples were injected with a split ratio of 1/100. Detector and injector temperatures were set at 260 ºC. The individual FA were identified by comparing their retention times with standards of FAME and quantified by using C21:0 as internal standard. Results were expressed as a percentage of the total FAME.

Statistical Analysis

Data were analyzed by least squares method using a model with line (with three levels, A, V and R) and age (with two levels, 9 and 13) effects. Residuals were calculated after line and age effects were

removed from the data. Residual correlations were computed applying the CORR procedure of SAS (SAS Institute Inc. Cary, USA, 2002).

RESULTS AND DISCUSSION

Correlation coefficients between PF content, lipogenic enzyme activities and FA composition of PF tissue are given in Table 1. Perirenal fat content showed a positive relationship with G6PDH and ME activities. Conversely, no relationship was found with FAS activity. Activities of the three lipogenic enzymes were positively correlated (r>0.59) as it was expected since the three enzymes are involved in PF accretion. Gondret *et al*. (2004) did not find any relationship between lipogenic activities and lipid content in PF in rabbits of age between 10 and 20 weeks. This could be related to the decrease of lipogenic activity in PF with age.

Table 1: Residual correlation coefficients among perirenal fat content, lipogenic enzyme activities and FA composition of perirenal adipose tissue

	PF	G6PDH	ME	FAS	SFA	MUFA	PUFA
PF	1	0.37*	0.28*	0.01	0.29*	0.33*	-0.55*
G6PDH		1	0.65*	0.59*	0.15	0.18	-0.30*
ME			1	0.63*	0.35*	-0.30*	-0.11
FAS				1	0.36*	-0.38*	-0.06
SFA					1	-0.38*	-0.69*
MUFA						1	-0.41*
PUFA							1

Perirenal fat content (PF) is expressed in g; Activities of lipogenic enzymes glucose-6-phosphate dehydrogenase (G6PDH), malic enzyme (ME) and fatty acid synthase (FAS) are expressed in nmol/min per g of tissue; FA composition is expressed as % of total FA, saturated FA (SFA), monounsaturated FA (MUFA) and polyunsaturated FA (PUFA); *Significant correlation at $P<0.05$.

An association between PF content and its FA composition was found, showing positive correlations between PF and SFA (r=0.29) and MUFA (r=0.33) and negative between PF and PUFA (r=-0.55). These findings are consistent with De Smet (2004), who pointed out in several species an increase in SFA and MUFA content and a decrease in PUFA with increasing fat content. There was a relationship between lipogenic activity and FA composition in PF tissue. Malic and FAS activities were positively correlated with SFA and negatively with MUFA percentage. The activity of G6PDH was negatively correlated with PUFA percentage. Glucose-6-phosphate dehydrogenase and ME provide NADPH required in FA biosynthesis and then, FAS catalyzes the synthesis of saturated long-chain FA from NADPH and acetyl-CoA (Nelson and Cox, 2000). To our knowledge, there is no information about the relationships between lipogenic enzymes and FA composition in rabbits. In pigs, Doran *et al.* (2006) found a positive correlation between stearoyl-CoA desaturase, a key lipogenic enzyme, and total fatty acid in muscle.

Table 2 shows correlation coefficients among IMF content, lipogenic enzyme activities, catabolic enzyme activities and FA composition of LM. No association was found between IMF content and lipogenic activity of LM. This could be related to the low lipogenic potential and lipid content of LM (0.71 g/100 g in Zomeño *et al*., 2010). Gondret *et al*. (1997) found strong and positive correlations between IMF of LM and G6PDH and ME (r=0.71 and r=0.70, respectively), but in this study IMF content was higher (0.8 to 2.2 g/100 g).

Semimembranosus proprius (SP) muscle is an oxidative muscle with higher lipid content and lipogenic capacity than LM (Gondret *et al*., 2009; Zomeño *et al*., 2010). Intramuscular fat content of SP is not available due to its small size. However, when we study the relationship between IMF of LM and lipogenic enzymes of SP muscle, we found positive correlations between these traits (r=0.52 for G6PDH and IMF, r=0.42 for ME and IMF and r=0.56 for FAS and IMF). These correlations are in line with those obtained in PF tissue (Table 1).

Table 2: Residual correlation coefficients among intramuscular fat content, lipogenic enzyme activities, catabolic enzyme activities and fatty acid composition of *Longissimus dorsi* muscle

	IMF	G6PDH	ME	FAS	LDH	HAD	CS	AL	NL	APL	SFA	MUFA	PUFA
IMF	1	0.13	-0.21	-0.20	0.21	-0.16	0.03	-0.23†	0.13	-0.04	0.35*	0.29*	-0.43*
G6PDH		1	-0.08	-0.14	-0.22	0.10	0.08	-0.31*	-0.04	-0.16	0.13	0.12	-0.17
ME			1	0.10	-0.15	0.34*	-0.13	0.17	0.16	-0.03	-0.22	0.16	-0.03
FAS				1	-0.17	0.07	-0.17	0.27†	0.20	-0.18	-0.04	0.09	-0.07
LDH					1	0.07	0.11	-0.12	0.12	-0.16	0.18	-0.26†	0.14
HAD						1	0.10	-0.14	0.09	-0.21	0.02	-0.03	0.01
CS							1	-0.15	-0.06	-0.21	-0.16	-0.01	0.08
AL								1	0.09	0.35*	-0.10	-0.07	0.11
NL									1	-0.27*	0.07	-0.08	0.04
APL										1	-0.12	0.20	-0.12
SFA											1	0.01	-0.49*
MUFA												1	-0.87*
PUFA													1

IMF, intramuscular fat content (g/100g); Activities of enzymes lactate dehydrogenase (LDH), β-hydroxyacyl-CoA dehydrogenase (HAD) and citrate synthase (CS) are expressed in mmol/min per g of tissue; Actitivities of lipolytic enzymes acid lipase (AL), neutral lipase (NL) and acid phospholipase (APL) are expressed in mmol/h per g of tissue; *Significant correlation at $P<0.05$; †Significant correlation at $P<0.10$.

In LM muscle, catabolic enzymes were also studied. No relationship was found between IMF and the activity of HAD, CS and LDH enzymes. However, IMF was negatively related to AL activity ($P<0.10$). In fact, muscle lipases are involved in degradation of TAG and subsequent generation of free FA (Toldrá, 2002). As in PF tissue, IMF content was related to FA composition. The percentage of SFA and MUFA increased with increasing IMF and the percentage of PUFA decreased. No relationship was found between lipogenic and FA composition, which can be related to the lower lipogenic potential of LM.

Regarding the relationship between anabolic and catabolic pathways, there was a negative relationship between G6PDH and lipolytic activity (AL). In line with this result, lipoprotein lipase was negatively associated with muscle lipogenesis (Gondret *et al*., 2004). On the contrary, ME showed a positive relationship with oxidative potential, with HAD activity in LM (Table 2) and with CS in SP muscle (r=0.28). Different results were obtained by Gondret et al. (2004) who found that enhanced muscle capacity for lipogenesis was balanced by a decreased oxidative catabolism (HAD and CS). However, the positive relationship between lipogenic and catabolic enzymes can be explained by the fact that tricarboxylic acid cycle not only serves in catabolic processes of FA, but also it provides precursors for many biosynthetic pathways (Nelson and Cox, 2000). No relationship was observed between glycolytic, oxidative and lipolytic activities in LM.

PF and IMF adipose tissues were not correlated (r=-0.02). This correlation was independent of line and age effects and it is in accordance with previous results (Hernández *et al*., 2010) showing an independence of metabolic capacity of IMF from other adipose tissues.

CONCLUSIONS

The results from this study showed a relationship between lipogenic activity and adipose tissue in perirenal fat as well as in IMF. The amount of PF content had an influence on its FA composition. A similar relationship was found between IMF content and its FA composition in muscle. We report some evidence of the coordinated use of anabolic and catabolic pathways for lipid deposition in muscle. The lack of relationship between PF and IMF tissues supports the idea of independence between adipose tissues and, therefore, suggests the possibility of increasing IMF without increasing overall adiposity carcass.

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REFERENCES

Clarke, S.D. 1993. Regulation of fatty acid synthase gene expression: an approach for reducing fat accumulation. *J. Anim. Sci., 71, 1957-1965.*

De Smet S., Raes K., Demeyer D. 2004. Meat fatty acid composition as affected by fatness and genetic factors: a review. *Anim. Res., 53, 81-98.*

Doran O., Moule S.K., Teye G.A., Whittington F.M., Hallet K.G., Wood J.D. 2006. A reduced protein diet induces stearoyl-CoA desaturase protein expression in pig muscle but not in subcutaneus adipose tissue: relationship with intramuscular lipid formation. *British J. Nutr., 95, 609-617.*

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

Gondret F., Hocquette J.F., Herpin P. 2004. Age-related relationship between muscle fat content and metabolic traits in growing rabbits. *Reprod. Nutr. Dev., 44, 1-16.*

Gondret F., Hernández P., Rémignon H., Combes S. 2009. Skeletal muscle adaptations and biochemical properties of tendons in response to jump exercise in rabbits. *J. Anim. Sci., 87, 544-553.*

Hernández P., Navarro J.L., Toldrá F. 1999. Effect of frozen storage on lipids and lipolytic activities in the longissimus dorsi muscle of pig. *Lebens. Unters. Forsch., 208, 110–115.*

Hernández P., Kačírková A., Juste V., Zomeño C., Blasco A. 2010. Genetic variability of lipogenic activity and lipid composition in rabbit adipose tissue. *In: Proc. 56th International Congress of Meat Science and Technology, 2010 August, Jeju, Korea.*

Jurie C., Cassar-Malek I., Bonnet M., Leroux C., Bauchart D., Boulesteix P., Pethick D.W., Hocquette J.F. 2007. Adipocyte fatty acid-binding protein and mitochondrial enzyme activities in muscles as relevant indicators of marbling in cattle. *J. Anim. Sci., 85, 2660-2669.*

Nelson D.L., Cox M.M. 2000. *Lehninger Principles of Biochemistry. Worth Publishers, New York, USA.*

O'Fallon J.V., Busboom J.R., Nelson M.L., Gaskins C.T. 2007. A direct method for fatty acid methyl ester synthesis: Application to wet meat tissue, oils, and feedstuffs. *J. Anim. Sci., 85, 1511-1521.*

SAS 2002. SAS/STAT User's guide (Release 9.1) *Statistical Analysis System Institute Inc., Cary NC, USA.*

Toldrá F. 2002. Characterization of lipolysis. In: *Dry-cured meat products. Food & Nutrition Press, Connecticut, USA, 135-152.*

Zomeño C., Blasco A., Hernández P. 2010. Influence of genetic line on lipid metabolism traits of rabbit muscle. *J. Anim. Sci., 88, 3419-3427.*