Associations Of A Bovine *SCD1* SNP With Fatty Acid Composition In Concentrate-Finished Yearling Bulls Of Different Muscular Hypertrophy Genotypes

C. Li[*,†], M. Vinsky[*], N. Aldai[*], K. Osoro[‡], M. E. R. Dugan[*]

Introduction

Accumulated evidence suggests that fat composition has a more profound impact on human health than the amount of fat in the diet (Woodside and Kromhout (2005)). Increasing the beneficial fat composition in beef can be achieved through diet modification (Moloney (2007)). However, even when steers are fed the same diet, significant variation in the fatty acid composition has been observed (Dugan et al. (2007)), indicating a great potential to improve the content of beneficial fatty acids through genetic selection. However, genetic evaluation and selection has not been practiced on fatty acids in cattle due to the complexity and cost of fatty acid analyses in various beef cuts. Therefore, identification of DNA markers or gene polymorphisms influencing fatty acid composition may facilitate the genetic improvement of fatty acid profiles through marker-assisted or genomic selection and/or marker-based diet management.

Stearoyl-CoA desaturase is an enzyme encoded by the stearoyl-CoA desaturase gene (*SCD1*) and plays a rate-limiting role in the synthesis of unsaturated fatty acids by insertion of a cis-double bond in the Δ9 position of fatty acid substrates (Kim and Ntambi (1999)). Taniguchi et al. (2004) detected a single nucleotide polymorphism (SNP) [C/T] in the coding region of bovine *SCD1* that causes an amino acid replacement from valine (type V) to alanine (type A), and found that the SNP allele C or type A was associated with higher percentage of mono unsaturated fatty acid (MUFA) in intramuscular fat of Japanese Black cattle. In this study, we examined the associations of the *SCD1* SNP with the fatty acid composition in subcutaneous (SC), intermuscular (IT) and intramuscular (IM) fat tissues of yearling bulls of northern-Spanish breeds with different muscular hypertrophy genotypes.

Materials and methods

Animals and management. Ninety nine yearling bulls of Asturiana de los Valles (AV) and Asturiana de la Montaña (AM) breeds were used in this study. The AV animals included double-muscled homozygote (mh/mh, n = 24), heterozygote (mh/+, n = 26) and normal homozygote (+/+, n = 24) depending on the status of muscular hypertrophy determined by genotyping the 11-bp deletion in the coding sequence of the *myostatin* gene (Grobet et al.

[*] Agriculture and Agri-Food Canada, Lacombe Research Centre, 6000 C&E Trail, Lacombe, Alberta, Canada T4L 1W1
† Department of Agricultural, Food and Nutritional Science, University of Alberta, Edmonton, Alberta, Canada, T6G 2P5;
‡Área de Sistemas de Producción Animal, Servicio Regional de Investigación Desarrollo Agroalimentario, Apdo. 13, 33300, Villaviciosa, Asturias, Spain

(1998)). The AM breed is characterized as being small to medium-sized animals and lack the mutation responsible for doubling muscling (n = 25). Animal management was described in a previous report (Aldai et al. (2007)). Briefly, animals were produced over a three-year period and were fed a concentrate diet (84% barley, 10% soybean meal, 3% soybean oil, 3% minerals, vitamins and oligoelements) and barley straw *ad libitum* for 230 days and slaughtered at an average live weight of 519 kg.

Animal tissue collection and fatty acid analyses. After slaughter (24h post-mortem), meat (longissimus thoracis) and adipose tissue (subcutaneous and intermuscular fat) were dissected from the 8th rib region, vacuum packaged and frozen at -80 °C. Prior to the fatty acid analysis, tissues were thawed and ground through a 3 mm plate and then 1 g muscle or 30 mg adipose tissue (subcutaneous and intermuscular) were saponified in KOH in methanol/water (50:50, v/v). Fatty acids were methylated using trimethylsilyl-diazomethane in methanol / toluene (2:1, v/v), and fatty acid methyl esters (FAME) were analyzed by GC using a CP Sil-88 100m column with FID detector (Aldai et al. (2006)). Fatty acid contents were expressed as a percentage of total FAME for individual or groups of fatty acids, and in total 54 individual and groups of fatty acids were analysed for each tissue including a health index (HI = (total MUFA + total PUFA) / (4 x C14:0 + C16:0) that was calculated as the inverse of atherogenic index as proposed by Ulbricht and Southgate (1991).

Genotyping and statistical analyses. Genotyping of the *SCD1* SNP was performed using the PCR-RFLP procedure as described by Taniguchi et al. (2004). In brief, PCR was performed with a forward (SCDF878, 5´-ATGTATGGATACCGCCCTTATGAC-3´) and a reverse primer (SCDR878, 5´-TTCTGGCACGTAACCTAATACCCTAAGC-3´) to produce a 145bp fragment. Following a 16 hour incubation at 37°C with 0.5U of Fnu4HI, samples were run on a 3% agarose gel with 0.5x TBE buffer and stained with ethidium bromide. Animal's genotypes were called as having a "T" allele when a 116bp and 29bp fragment were present and/or having a "C" allele when 68bp, 48bp and 29bp fragments were visible. Association analyses were performed using the GLM procedure of SAS (SAS Institute Inc. (2001). Cary, NC. USA) with fixed effects including year, *myostatin* genotype, *SCD1* SNP genotype, interaction between the *myostatin* and *SCD1* SNP genotypes, and age of slaughter as a covariate.

Results and discussion

Significant associations of the *SCD1* SNP were detected for three MUFA, which included 9c-14:1 in all the three fat tissues, 9c-16:1 in both the IM and SC tissues, and 13c-18:1 in the SC fat. The SNP was also found significantly associated with conjugated linoelic acid (CLA) (9c,11t-18:2) and the total CLA (9c,11t-/7t,9c-/8t,10c-/10t,12c-18:2) in the IM fat ($P<0.05$) (Table 1). The predominant association of the *SCD1* SNP with fatty acids containing a cis-9 double bound is in agreement with role of the *SCD1* gene in the desaturation of fatty acid substrates in the Δ9 position. However, the *SCD1* SNP was not significantly associated with the total MUFA in any of the three tissues (P values ranged from 0.31 to 0.61), although it was significantly associated with 13c-18:1 in the SC fat. It was found that the "C" allele of the *SCD1* SNP or type A was significantly associated with a higher level of MUFA 9c-14:1 in all the three fat tissues, higher level of 13c-18:1 in the SC fat tissue but lower lever of 9c-16:1 in both the IM and SC tissues (Table 1). The opposite association of the *SCD1* SNP

allele on different types of MUFA may contribute to the non-significant association with the total amount of MUFA. The result also suggests that other *SCD1* SNPs or genes may be involved in the desaturation of fatty acids.

The effect of the muscular hypertrophy on the fatty acid composition of the three tissues was reported previously (Aldai et al. (2007)). In general, the genotype effects on the fatty acid profiles were more evident in the IM tissue, and animals carrying mh/mh genotypes had lower percentages of total fat, saturated fat, MUFA and total CLA. In this study, we identified significant interaction effects between the genotypes of the *SCD1* and *myostatin* genotypes on three types of MUFA and one saturated fatty acid, which included 11c-18:1 and 12c-18:1 in the IT fat tissue and 9c-18:1, 19:0 in the SC tissue (Table 2). Further multiple comparisons of the least square means between the genotype combinations revealed that the genotype combinations had significant effects on the fatty acid components (Table 2). For example, the "TT" genotype of the *SCD1* SNP was associated with a lower level of 11c-18:1 in the presence of mh/-- genotype, which suggests that the *myostatin* genotype may influence the effect of the *SCD1* SNP on the desaturation of fatty acids. However, further study is needed to ascertain whether and how *myostatin* gene interacts with *SCD1* in the biosynthesis of MUFA in beef cattle.

Table 1. Associations of the *SCD1* SNP with fatty acids in the intramuscular (IM), subcutaneous (SC) and intermuscular (IT) adipose tissues in concentrate fed yearling bulls of Asturiana de los Valles (AV) and Asturiana de la Montaña (AM) breeds‡.

Fatty acids[a]	Tissue	*SCD1* SNP genotype (N)[b]			Additive effect [c]	Dominance effect [d]
		CC (29)	CT (51)	TT (19)		
9c-14:1	IM	0.42±0.04	0.36±0.03	0.24±0.04	0.09±0.02***	0.03±0.03
9c-14:1	IT	0.57±0.06	0.54±0.05	0.28±0.07	0.14±0.04***	0.12±0.05*
9c-14:1	SC	1.11±0.08	1.01±0.07	0.76±0.09	0.17±0.05***	0.07±0.06
9c-16:1	IM	2.11±0.13	2.32±0.10	2.48±0.14	-0.19±0.07*	0.02±0.09
9c-16:1	SC	4.00±0.20	4.33±0.16	4.54±0.22	-0.27±0.12*	0.06±0.15
13c-18:1	SC	0.25±0.02	0.26±0.01	0.20±0.02	0.02±0.01*	0.03±0.01*
9c,11t-18:2	IM	0.15±0.02	0.18±0.02	0.22±0.02	-0.04±0.01**	0.01±0.02
Total CLA	IM	0.17±0.02	0.21±0.02	0.25±0.02	-0.04±0.01**	0.01±0.02
MUFA	IM	32.06±0.94	33.28±0.75	32.91±1.01	-0.42±0.56	0.79±0.68
MUFA	SC	42.77±081	43.49±0.65	42.63±0.87	0.07±0.48	0.79±0.58
MUFA	IT	39.44±0.87	40.22±0.69	39.48±0.94	-0.02±0.52	0.79±0.62

‡ Results were presented only for fatty acids showing significant associations with the SNP ($P<0.05$) except for MUFA.

[a] Total CLA includes 9c,11t-/7t,9c-/8t,10c-/10t,12c-18:2.

[b] Least square means ± standard error for genotypes CC, CT and TT. The counts of the genotype CC, CT and TT are in parentheses. Intralocus SNP genotypic frequencies conformed to Hardy-Weinberg equilibrium proportions at $P>0.05$.

[c] Estimated as the difference between means of the two homozygous genotypes divided by two.

[d] Estimated by subtracting the average of solutions for homozygous genotypes from that for heterozygous genotype.

* $P < 0.05$, ** $P<0.01$, *** $P<0.001$.

Table 2. Significant effects of the *SCD1* SNP and *myostatin* genotype combinations on fatty acids in the intramuscular (IM), subcutaneous (SC) and intermuscular (IT) adipose tissues in concentrate fed yearling bulls of AV and AM breeds‡.

SCD1: Myostatin	Count [a]	*11c-18:1* (IT)	*12c-18:1* (IT)	*9c-18:1* (SC)	*19:0* (SC)
CC:mh/mh	9	1.34±0.05a	0.33±0.02ab	21.97±1.03a	0.20±0.02ab
TC:mh/mh	15	1.38±0.04a	0.40±0.02b	24.22±0.79ab	0.23±0.01ab
CC: mh/--	5	1.35±0.07a	0.38±0.03ab	26.71±1.37abc	0.29±0.02b
TC: mh/--	15	1.41±0.04a	0.36±0.02ab	26.87±0.80bcd	0.23±0.01ab
TT: mh/--	6	1.03±0.07b	0.32±0.03ab	29.54±1.35cd	0.16±0.02a
CC: --/--	12	1.34±0.05a	0.32±0.02ab	24.95±0.88abc	0.21±0.02ab
TC: --/--	9	1.41±0.05a	0.34±0.02ab	27.13±1.04bcd	0.26±0.02ab
TT: --/--	2	1.51±0.11a	0.40±0.05b	25.16±2.16abc	0.20±0.04ab
CC: AM	2	1.42±0.12a	0.31±0.05ab	33.49±2.19d	0.18±0.04ab
TC: AM	12	1.37±0.05a	0.30±0.02a	29.30±0.98cd	0.22±0.02ab
TT: AM	11	1.42±0.05a	0.35±0.02ab	27.65±1.00bcd	0.20±0.02ab

‡ Each combination of the *SCD1* SNP and the *myostatin* genotypes is presented with least square means ± standard error of the fatty acids. Least square means with different letters within a column are significantly different at P<0.05 of Tukey-Kramer adjustment by the multiple comparison procedure.
[a] Counts of each genotype combination with TT:mh/mh absent.

Conclusion

The SNP [C/T] in the coding region of bovine *SCD1* gene was predominantly associated with individual types of MUFA and CLA including total CLA containing a cis-9 double bond in one or more fat tissues, but not with the total amount of MUFA. Significant interaction effects between the *SCD1* SNP and muscular hypertrophy genotypes were also identified on certain individual types of MUFA and saturated fatty acids in some fat tissues.

Acknowledgements

The work is part of the AAFC Matching Investment Initiative (MII) project to Li. C. Noelia Aldai acknowledges a Marie Curie Fellowship (OIF) awarded by the 7th European Community Framework Program.

References

Aldai, N., Najera, A.I., Dugan, M.E.R. *et al.* (2007). *Meat Sci*. 76: 682-691.
Aldai, N., Osoro, K., Barron, L.J.R. *et al.* (2006). *J. Chromatogr. A* 1110: 133-139.
Dugan, M. E., Kramer, J. K., Robertson, W. M. *et al*. (2007). *Lipids*. 42(6): 509-18.
Grobet, L., Poncelet, D., Royo, J.L.*et al.* (1998). *Mamm. Genome* 9: 210-213.
Kim, Y-C. and Ntambi, J. M. (1999). *Biochem. Biophys. Res. Commun*. 266:1-4.
Moloney, A. P. (2007). *Irish Vet. L.* 60(3):180-185.
Taniguchi, M., Utsugi, T., Oyama, K. *et al*. (2004). *Mammalian Genome*. 14:142-148.
Ulbricht, T.L.V. and Southgate, D.A.T. (1991). *The Lancet* 8773: 985-992.
Woodside, J.V. and Kromhout, D. (2005). *Proc Nutr Soc*. 64(4):554-64. Review.