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Diacylglycerol acyltransferase (DGAT) activity in relation to muscle fat content and *DGAT1* genotype in two different breeds of *Bos Taurus* (short communication)

Abstract

To circumvent the obvious waste of resources when producing good marbled beef, research is needed to clarify mechanisms which are involved in intramuscular fat storage in cattle. The possible association between the activity of diacylglycerol acyltransferase (DGAT) and muscle fat content was examined in samples of *longissimus dorsi* (LD) and *semitendinosus* (ST) muscle from Holstein and Charolais bulls. The Holstein bulls exhibited higher fat content in both muscles and higher marbling score. In Holstein, DGAT activity was enhanced in the LD muscle, and there was a tentative positive relationship between DGAT activity and the fat content in ST muscle. When muscle DGAT activity was examined as a function of *DGAT1* genotype for all animals, regardless of breed, the DGAT activity of LD muscle of the K/K genotype was about five-fold greater than for either the K/A or A/A genotypes. Further investigation on the relationship between *DGAT1* genotype and i.m. fat is required before this may be developed as a selection tool for marbling.

Key Words: triacylglycerol synthesis; intramuscular fat; marbling; cattle

Zusammenfassung

Titel der Arbeit: **Diacylglycerol-Acyltransferase (DGAT)-Aktivität in Beziehung zum Muskelfettgehalt und zum *DGAT1*-Genotyp in zwei unterschiedlichen Rassen von *Bos taurus*** (Kurzmitteilung)

Um die Verschwendung von Ressourcen bei der Erzeugung von gut marmoriertem Rindfleisch zu vermeiden, sind die Mechanismen der intramuskulären Fetteinlagerung zu erforschen. Die mögliche Beziehung zwischen der DGAT-Aktivität und dem Muskelfettgehalt wurde in Proben vom *M. longissimus dorsi* (LD) und *M. semitendinosus* (ST) von Holstein- und Charolaisbullen untersucht. Die Holsteinbullen zeigten in beiden Muskeln einen höheren Fettgehalt und höhere Punktzahlen für die Marmorierung. Bei den Holsteinbullen war die DGAT-Aktivität im LD erhöht, und im ST scheint ebenfalls eine positive Beziehung zwischen diesem Enzym und dem Fettgehalt dieses Muskels zu bestehen. Wenn die DGAT-Aktivität im Muskel zusammen mit dem *DGAT1*-Genotyp für alle Tiere, ungeachtet der Rassen betrachtet wird, findet man im Vergleich mit dem K/A- oder A/A-Genotyp eine fünfmal höhere DGAT-Aktivität im K/K-Genotyp. Weitere Untersuchungen über die Beziehung zwischen dem *DGAT1* Genotyp und dem intramuskulären Fettgehalt ist notwendig, bevor die DGAT-Aktivität als Selektionskriterium für die Marmorierung genutzt werden kann.

Schlüsselwörter: Triglyceridsynthese; intramuskuläres Fett; Marmorierung; Rind

Introduction

Marbling is a visual evaluation of the amount of intramuscular fat deposition, and is a desirable trait to the consumer. The degree of marbling varies among and within breeds. A greater understanding of the mechanisms behind these differences could lead to the development of molecular and biochemical markers for use in selection strategies to enhance marbling. Fat deposition is largely a result of hypertrophy of adipocytes, (i.e. fat cells), which is accompanied by increased storage lipid accumulation. Diacylglycerol acyltransferase (DGAT, EC 2.3.1.20) catalyzes the

terminal step in triacylglycerol (TAG) synthesis via the acyl-CoA-dependent acylation of *sn*-1,2-diacylglycerol and the level of DGAT activity may have a substantial effect on the quantity of triacylglycerol (TAG) deposited in fat-forming tissues (MAYOREK et al., 1989). Recently, a non-conservative K232A substitution in DGAT1 was associated with alterations in the lipid content of *semitendinosus* (ST) muscle in cattle (THALLER et al., 2003). *In vitro* assays in a baculovirus expression system indicated that DGAT1 was more catalytically active with the lysine residue at position 232 when compared with the protein with an alanine substitution at the same position (GRISART et al., 2004). MIDDLETON et al. (1998) found a negative correlation between lipid content of the *pars costalis diaphragmatis* muscle and DGAT activity within this tissue in Japanese Black hybrid cattle, which suggested DGAT was down-regulated as this depot became larger. The current study continues to investigate this relationship in the *longissimus dorsi* (LD) and ST muscles of Holstein and Charolais bulls, which produce considerably less lipids in muscle. Both DGAT activity and *DGAT1* genotype were taken into consideration in this investigation.

Materials and Methods

Animals

The animals used in this study were the P₀ generation of the segregating families (SEGFAM) herd, currently under investigation at the Research Institute for the Biology of Farm Animals in Dummerstorf (KÜHN et al., 2002). Starting at the age of seven days, all calves were raised on a milk replacer diet under standardized conditions until 120 days of age. From the fifth to the 18th month of age, the animals were tethered on individual feeding places with semi-slatted floor and automatic drinking bowls. The ration was offered *ad libitum* and was composed of a concentrate (44.8 % barley, 36.9 % molasses-chips, 13.7 % soybean extraction meal, 3 % molasses, and 1.6 % mineral and vitamin premix) and chaffed hay in the proportion 3:1 resulting in an energy content of 11.7 MJ ME/kg DM feed (BELLMANN et al., 2004). Eighteen Holstein bulls and 18 Charolais bulls were slaughtered at 18 mo of age. Muscle tissue from LD and ST muscle was collected within 30 min after slaughter. Samples were immediately frozen in liquid nitrogen, and stored at -70°C.

Determination of muscle lipid content, microsome preparation and DGAT activity assay

The lipid content of LD and ST whole muscle was obtained via the Soxhlet extraction (Association of Official Agricultural Chemists, 1984). Marbling scores were assigned based on the German standard using photographic standards in a six-point scale with 1 being extremely low marbled and 6 being extremely high marbled. Microsomal fractions were prepared from muscle tissue as described in LOZEMAN et al. (2001), except that 50 mM HEPES (pH 7.4) was used. [1-¹⁴C] Oleoyl-CoA was synthesized from radiolabelled fatty acids as described by Taylor et al. (1990). The microsome was assayed for DGAT activity as previously described by LOZEMAN et al. (2001) based on the incorporation of [1-¹⁴C] Oleoyl-CoA into TAG. Two trials were performed, each with four to six replicates for each animal and muscle.

Genotyping at the *DGAT1* K232 mutation

To account for the effects of the *DGAT1* K232A alleles previously shown to exhibit an effect on intramuscular fat content (THALLER et al., 2003) and on *in vitro* efficiency of TAG synthesis (GRISART et al., 2004), all individuals were genotyped for the non-conservative *DGAT1* K232A mutation according to WINTER et al. (2002).

Statistical analysis

The median DGAT activity of each animal and muscle was calculated for each trial and then averaged. The CORR and TTEST procedures for SAS Windows Version 8 (SAS Institute Inc., 1999) were used in statistical analyses. A linear model was applied (PROC GLM, SAS Institute Inc., 1999) for the analysis of the DGAT activity, which included fixed effects of breed, sire of the individuals and *DGAT1* genotype.

Results

Muscle DGAT activity, muscle lipid content and marbling score

Data for Charolais and Holstein are presented in Table 1. Holstein bulls had a higher ($P < 0.05$) percentage of lipid and higher ($P < 0.10$) DGAT activity in the LD muscle than Charolais. Although Holstein had a slightly greater ($P < 0.10$) percent lipid content in ST muscle than Charolais, there were no breed differences in DGAT activity for this muscle. Marbling scores were higher ($P < 0.001$) for Holstein than Charolais. In both Charolais and Holstein, DGAT activity was higher in LD muscle than in the ST muscle ($P < 0.05$ and $P < 0.001$, respectively). The percentage of lipid in LD muscle was also higher than for ST muscle in Charolais and Holstein bulls ($P < 0.05$ and $P < 0.001$, respectively).

Table 1

Mean and standard deviation (SD) for muscle DGAT activities and fat related characteristics in Charolais and Holstein bulls (Mittelwerte und Standardabweichungen (SD) für die DGAT-Aktivität und die Fettmerkmale im Muskel von Charolais- und Holsteinbullen)

	Charolais (Mean \pm SD)	Holstein (Mean \pm SD)
DGAT activity in LD muscle (pmol TAG min ⁻¹ mg ⁻¹)	44.8 ^{h,j} \pm 32.5	74.9 ^{c,l} \pm 64.5
DGAT activity in ST muscle (pmol TAG min ⁻¹ mg ⁻¹)	21.0 ^k \pm 19.7	16.5 ^m \pm 10.4
Marbling score in LD muscle ^a	2.1 ^d \pm 0.6	2.8 ^e \pm 0.6
Lipid content in LD muscle (%)	2.6 ^{f,n} \pm 1.8	4.1 ^{g,p} \pm 1.7
Lipid content in ST muscle (%)	1.4 ^{h,o} \pm 0.9	1.9 ^{i,q} \pm 0.9

^a as determined by German grading standards

^{b,c,d,e,f,g,h,i,j} Means with these pairs of superscripts within the same row differ significantly

^{bc} ($P < 0.10$), ^{de} ($P < 0.001$), ^{fg} ($P < 0.05$) ^{hi} ($P < 0.10$)

^{j,k,l,m,n,o,p,q} Means with these pairs of superscripts within the same column differ significantly

^{jk} ($P < 0.05$), ^{lm} ($P < 0.001$), ^{no} ($P < 0.05$), ^{pq} ($P < 0.001$)

Associations involving muscle DGAT activity and fat-related characteristics of muscle

Pearson's correlation coefficients were calculated to examine possible relationships involving DGAT activity and lipid content of the investigated muscles (Table 2). In Charolais, positive associations were observed for the lipid content of LD ($P < 0.001$) and ST ($P < 0.001$) muscle in relation to marbling score. The DGAT activity of both muscle types showed no significant associations with muscle fat characteristics in Charolais.

In Holstein, the lipid content of LD muscle also showed a positive association ($P < 0.01$) with marbling score (Table 2). The lipid content of ST muscle in this case did not show a significant association with marbling score. In Holstein, however, there appeared to be a positive relationship ($P < 0.10$) between DGAT activity and the lipid content of ST muscle.

Table 2

Pearson correlation coefficients for muscle DGAT activity and fat related characteristics in Charolais and Holstein bulls (Korrelationskoeffizienten nach Pearson für die DGAT-Aktivität und die Fettmerkmale im Muskel von Charolais- und Holsteinbullen)

	Lipid content		DGAT activity	
	ST muscle	LD muscle	LD muscle	ST muscle
Marbling score				
Charolais	0.78***	0.72***	0.32	-0.10
Holstein	0.25	0.65**	0.07	0.11
Lipid content in ST muscle				
Charolais		0.63**	0.22	-0.12
Holstein		0.36	0.20	0.41 [†]
Lipid content in LD muscle				
Charolais			-0.18	-0.04
Holstein			0.03	0.14
DGAT activity in LD muscle				
Charolais				-0.38
Holstein				0.29

[†] $P < 0.10$, ** $P < 0.01$, *** $P < 0.001$

Muscle DGAT activity in relation to DGAT1 genotype

Muscle DGAT activity versus *DGAT1* genotype is shown for all bulls, regardless of breed, in Table 3 marked differences were observed for enzyme activity between the two muscles. For LD muscle, the DGAT activity of the K/K *DGAT1* genotype was about five-fold greater than for either the K/A or A/A genotype ($P = 0.004$). In contrast, no differences in DGAT activity among all three genotypes were observed in ST muscle.

Table 3

Muscle DGAT activity (LSmeans, S.E. in brackets) in individuals with different genotypes for the non-conservative *DGAT1* K232A mutation (DGAT-Aktivität im Muskel (LSmeans, S.E. in Klammern) bei Individuen mit unterschiedlichem Genotyp bezüglich der non-conservativen *DGAT1* K232A Mutation)

	Genotype			<i>P</i> value for an effect of the genotype
	K/K n = 4	K/A n = 13	A/A n = 19	
DGAT activity in LD muscle (pmol TAG min ⁻¹ mg ⁻¹)	184.91 (36.78)	35.31 (15.44)	38.53 (13.98)	0.004
DGAT activity in ST muscle (pmol TAG min ⁻¹ mg ⁻¹)	21.12 (12.63)	14.65 (5.84)	18.64 (5.28)	0.795

Model: phenotype = breed + sire + genotype

Discussion

We found only a tentative relationship ($P < 0.10$) between DGAT activity and the lipid content of ST muscle from Holstein. No significant relationships existed in Charolais, or in the LD muscle of Holstein. This appears to contradict the strong negative

correlation between DGAT activity and lipid content found by MIDDLETON et al. (1998), although it should be reinforced that different breeds and muscles were used. For preparation of microsomes, MIDDLETON et al. (1998) were able to dissect i.m. fat from true muscle tissue, while this was not feasible in our study as there were only minimal amounts of visible i.m. fat present in the samples. Since the muscle of Japanese Black hybrid cattle accumulates substantially greater levels of i.m. fat compared to muscles of Charolais and Holstein, DGAT may be down-regulated at an earlier stage in the development of the Japanese Black hybrid cattle.

The observation of an increased DGAT activity in the LD muscle in individuals with the *DGAT1* K/K genotype is in agreement with the observation of the increased *in vitro* efficiency for TAG synthesis catalyzed by DGAT1 carrying a lysine residue at position 232 compared to the allele product which has an alanine residue at this position. The two alleles of *DGAT1*, which encode gene products differing in catalytic efficiency, were first reported in studies of milk fat production in dairy cattle (GRISART et al., 2002, 2004; WINTER et al., 2002). It should be noted, however, that there were no significant differences in DGAT activity between heterozygotes K/A and homozygous A/A individuals in the current study. A similar observation was described for the effects of *DGAT1* genotypes on lipid content of muscle (THALLER et al., 2003) and was interpreted as a potentially recessive gene effect. There were also marked differences observed between DGAT activity in LD and ST muscle in relation to *DGAT1* genotype. Due to the low number of homozygous K/K bulls in our study, further investigation of the effects of the *DGAT1* genotypes is required. It is possible that the *DGAT1* genotype, rather than DGAT activity, might be a useful tool in breeding for high marbling cattle.

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