Effects of body condition scores and fat depots on gene expression of fatty acid synthase in horses

L.A. Doran[1], R. Yemm[1], C.E. Dickinson[2], and K.L. Hossner[1] [1]

Department of Animal Science, Colorado State University, Fort Collins 80523[2] Department of Clinical Sciences, Veterinary Teaching Hospital, Colorado StateUniversity, Fort Collins 80523

ABSTRACT: The current study was designed to examine the expression of fatty acid synthase (FAS) genes in horse adipose tissue. Subcutaneous and visceral fat samples were collected from Quarter horses with obese or normal body condition scores and four fat samples were taken from clinical admission horses that were diagnosed with Cushing's disease at the C.S.U. Veterinary Teaching Hospital. Total RNA was isolated from the fat samples and analyzed by ribonuclease protection assay. A nonisotopic ribonucleic probe for fatty acid synthase (FAS) mRNA was constructed from sheep RNA and was utilized for analysis. The results from the ribonuclease protection assays on the four horses with both visceral and subcutaneous fat samples were analyzed to determine the effects of body condition scores and fat depots on the level of adipose tissue FAS mRNA expression. No difference in FAS gene expression was seen in horses with normal (BCS=3) or obese (BCS=5) body condition scores. The expression of FAS in visceral versus subcutaneous fat depots was numerically but not statistically significant. The data suggest that an ovine probe for FAS mRNA can be effectively used in horses. Although no differences were observed between fat depots, body condition score or Cushing's horses, use of larger sample numbers may provide significant results.

Introduction

Fatty acid synthase (FAS) is a lipogenic enzyme that catalyzes the irreversible and rate-limiting steps of lipogenesis (Abu-Elheiga et al., 2001). In the liver, the rate of FAS gene transcription dictates the level of FAS mRNA, whereas the FAS mRNA content of adipose tissue seems to be determined by factors that affect gene transcription and mRNA stability (Clarke, 1993). The FAS protein is primarily regulated by controlling the rate of its gene's transcription and is expressed in adipose tissue or liver (Prior and Scott, 1980).

Studies conducted by Prior and Scott (1980) and Lee and Hossner (2002) indicated that nutritional and hormonal regulation affect FAS gene expression in ruminants. Genes coding for lipogenic enzymes, such as FAS, are strongly influenced by insulin and glucocorticoids. It has been shown that hyperlipogenic response in rats is prevented by adrenalectomy (Williams and Berdanier, 1982).

Cushing's Syndrome is caused by a malfunction of the adrenal gland causing release of excessive amounts of the glucocorticoid, cortisol (Findling, 2005). Hepatic FAS expression in rodents is stimulated by glucocorticoids leading to increased adipose deposition (Clarke, 1993). One of the major symptoms of Cushing's syndrome is reduced ability to convert fat, carbohydrates and proteins into energy (Findling, 2005). Horses suffering from Cushing's disease, excess secretion of adrenocortical steroids, are characterized by, among other things, additional fat deposition. Levels of FAS expression in 3 horses diagnosed with Cushing's disease were also examined.

The current study was designed to examine expression of the FAS gene in normal or obese horses, in visceral and subcutaneous adipose tissue and in horses displaying symptoms of Cushing's syndrome using ribonuclease protection assays.

Materials and Methods

Subcutaneous (tailhead) and visceral adipose tissue samples were collected from Quarter horses from the Colorado State University Veterinary Teaching Hospital. The horses had body condition scores of 3 (normal) and 5 (obese). Four fat samples were also taken from clinical admission horses that were diagnosed with Cushing's disease at C.S.U. Veterinary Teaching Hospital. Total RNA was isolated from the adipose tissue samples using Trizol reagent (Sigma, St. Louis, MO). A nonisotopic ribonuclease protection assay was performed as described by Lee and Hossner (2002). The outline of this procedure follows. Antisense biotin-labeled riboprobes for FAS and 18S rRNA housekeeping genes were prepared from sheep adipose tissue RNA using reverse transcription and polymerase chain reaction with primers as described by Lee and Hossner (2002). FAS primers result in a product of 218 base pairs and 18S results in a product of 80 base pairs. During *in vitro* transcription, antisense biotin-labeled riboprobes were incubated with DNase I for 15 min at 37°C, transcripts were precipitated using 115 µl H2O, 15 µl ammonium acetate, and 300 µl of 100% ethanol, washed with 300 µl of 70% ethanol and resuspended in 20 µl DEPC-treated water. Ten micrograms of total horse RNA and 2 ng of biotin-labeled riboprobe were hybridized overnight at 45°C and then digested with RNase A and RNase T1. Following electrophoresis in 8 M urea and 5% polyacrylamide, the RNA was electrophoretically transferred onto a positively charged nylon membrane (Schleicher & Schuell, Keene, N.H.) with 0.5X TBE at 400 mA for 40 min using a semi-dry transblot apparatus (BioRad, Hercules, CA.). The molecular weight standard used in electrophoresis was Brightstar Biotinylated RNA Century Size Marker Plus from Ambion, which has standards at 1000, 750, 500, 400, 300, 200 and 100 base pairs. Protected FAS RNA was visualized by incubation with alkaline-phosphatase-conjugated strepavidin, incubated with CDP-star and exposed to CL-X Posure film for 30 min at room temperature. The 18S mouse rRNA probe, supplied with the Ambion RPA kit and resulting in a product of 80 base pairs, was used as the control probe in this study. After scanning the autoradiographs and analyzing the image using Scion Image software (NIH, Frederick, Maryland), a ratio was calculated for the intensity of the target band vs. 18S standard bands from each sample lane of the autoradiograph. The ratios were compared between samples from the four horses with both subcutaneous and visceral tissue available.

Gene		Primer Sequence	PCR condition* (Product size)
FAS	S	5'-ATGCCAGCGGGAAGCGTGTGATG-3'	94/1-64/1-72/1
	AS	5'-CCAAGCTTCTAATACGACTCACTATAGGGCCGCCAGAACCCGAGTGAATGAG-3'	(218 bp)

*Denaturation-annealing-elongation (temperature/minute).

Figure 1. The sequences of sense (S) and antisense (AS) primers used for synthesis of fatty acid synthase (FAS) probe. The minimal T7 promoter region (27 nt) is underlined (Lee and Hossner 2002).

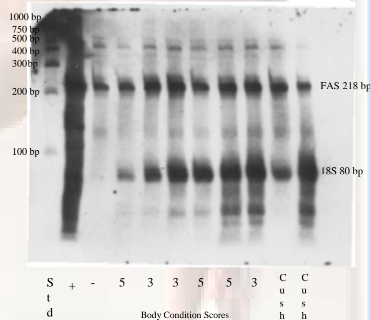

Figure 3. Fatty Acid Synthase mRNA expression in subcutaneous fat depots in horses. Results from a non-isotopic ribonuclease protection assay comparing FAS (218 bp) mRNA expression in horses of normal (3) body condition score, obese (5) body condition score and Cushing's syndrome. An antisense ribonucleic probe for 18S mRNA housekeeping gene with a product of 80 base pairs was also run as an internal control. The Biotinylated RNA Century Size Markers Plus from Ambion with standards at 1000, 750, 500, 400, 300, 200 and 100 was used to calculate the estimated actual base pairs for FAS and 18S (Figure 2).

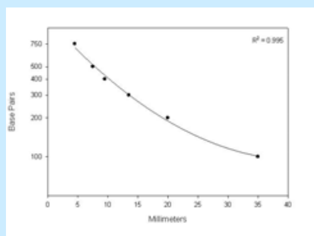

Figure 2. Denaturing polyacrylamide gel electrophoresis standard curve. A standard curve was created by measuring the distance the molecular weight markers traveled compared to their given number of base pairs. The BrightStar Biotinylated RNA Century Size Markers Plus from Ambion provided standards at 1000, 750, 500, 400, 300, 200 and 100 base pairs. When plotted agains the curve, the fitted size base pairs of FAS was 208 compared to the estimated base pairs of 218.

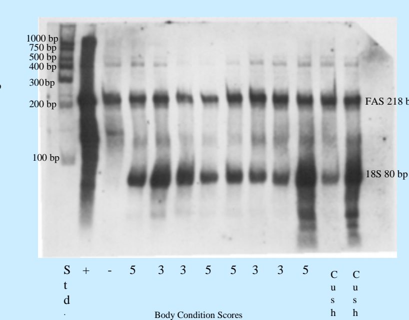

Figure 4. Fatty Acid Synthase mRNA expression in visceral fat depots in horses. Results from a non-isotopic ribonuclease protection assay comparing FAS (218 bp) mRNA expression in horses of normal (3) body condition score, obese (5) body condition score and diagnosed with Cushing's syndrome. An antisense ribonucleic probe for 18S mRNA housekeeping gene with a product of 80 base pairs was also run as an internal control. The Biotinylated RNA Century Size Markers Plus from Ambion with standards at 1000, 750, 500, 400, 300, 200 and 100 was used to calculate the estimated actual base pairs for FAS and 18S (Figure 2).

	BCS=3 (4)	BCS=5 (4)	All (8)
Tailhead fat (4)[1]	1.15 (0.21)[2]	0.68 (0.02)	0.92 (0.16)
Visceral fat (4)	0.36 (0.10)	0.69 (0.12)	0.52 (0.11)
All fat depos (8)	0.76 (0.25)	0.68 (0.05)	0.72 (0.12)

Table 1. Comparison of FAS expression in horses with different body condition scores. Average ratios of targeted FAS gene to 18S from the ribonuclease protection assay on four horses with both tailhead and visceral adipose tissue samples. Data compares the relative FAS gene expression in subcutaneous adipose tissue, visceral adipose tissue, body condition score of 3 (normal) and body condition score of 5 (obese). [1](Number of animals). [2] Average ratio (Standard Error).

Condition	Unaffected (8)	Cushing's (4)
FAS Expression	0.72 (0.12)	0.87 (0.32)

Table 2. Comparison of FAS expression in unaffected horses and horses with Cushing's syndrome. Shown are means +/- (standard errors) of ratios of FAS to 18S. Results are from 8 adipose tissue samples from unaffected horses and four adipose tissue samples from horses with Cushing's syndrome.

Results

Attempts to prepare the nonisotopic antisense riboprobes using the ovine primers for FAS, as presented in Figure 1 (Lee and Hossner 2002), with equine RNA were unsuccessful. However, ovine primers were used successfully on ovine RNA, creating a working riboprobe that successfully hybridized with horse RNA. The predicted length for FAS was 218 base pairs. We calculated an actual length of 208.3 base pairs using a standard curve that compares distance traveled to number of base pairs (Figure 2). 18S has a predicted length of 80 base pairs. We were not able to calculate an actual length for 18S because it fell outside of our molecular weight standards (Figure 2).

No differences were observed in FAS mRNA levels between subcutaneous and visceral fat depots. In addition, there were no differences in FAS gene expression in horses with a body condition score of 3 (normal) to a body condition score of 5 (obese) (Table 1). There was a numerical trend that suggested that FAS mRNA may be expressed at a higher level in subcutaneous fat than in visceral fat, but further studies with a greater number of animals would be necessary to obtain substantive results.

The visceral and tailhead fat from three horses with Cushing's syndrome was also analyzed. No differences in FAS gene expression were noted within the fat depots of these animals or between these horses and their unaffected counterparts (Table 2).

Discussion

The contribution of hepatic fatty acid synthesis to total carcass fat storage varies with species. Fatty acid synthesis in ruminant liver is minimal, while it accounts for more than 90% of total fatty acid synthetic capacity in chickens (Clarke, 1993). Due to this variation in FAS gene expression in fat depots among species, we investigated the expression of fatty acid synthase in visceral fat versus subcutaneous fat in horses. We did not observe any differences in FAS expression in adipose depots. However, further studies with a larger number of samples would need to be conducted before significant conclusions could be drawn. There is a numerical trend that indicates that FAS gene expression is higher in subcutaneous fat than in visceral fat (Table 1).

FAS expression in equine Cushing's syndrome has never been studied. The ability to quantitate and standardize the levels of FAS expression in horses could lead to a reliable test for diagnosing Cushing's syndrome. Once FAS expression in horses is understood, efforts to regulate and overcome the symptom of excessive fat storage due to Cushing's syndrome.

Our results showed no difference between the horses with Cushing's disease and the unaffected horses (Table 2). However, our experimental design was severely limited in the availability of equine visceral and subcutaneous adipose tissue samples.

.Despite inconclusive results due to the small sample size, we established that a nonisotopic antisense riboprobe for the ovine fatty acid synthase could be used successfully to examine the FAS mRNA in equines. The antisense riboprobes were constructed from sheep FAS primers and sheep total RNA. Although primers were ineffective when equine RNA was used, the ovine probes hybridized successfully with the equine total RNA. This suggests that the overall gene sequence for FAS in horses is similar to FAS in sheep. Now that it has been verified that sheep FAS primers are effective with horse RNA and ribonuclease protection assays can be performed, further analysis of the effects of fat depots and body condition scoring on FAS gene expression in relation to Cushing's syndrome can be performed.

The current study provides information on the similarities of fatty acid synthase between sheep and horses and outlines a reliable process by which to measure the amounts of FAS gene expression in horses.

Implications

The current study demonstrates that a nonisotopic antisense riboprobe for the ovine fatty acid synthase can be used successfully to quantitate the FAS mRNA expression in equines. Application of this information will allow for further research into the effects of different body condition scores and fat depots on FAS mRNA expression and the role of fatty acid synthase in Cushing's syndrome in horses.

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