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Lack of genetic association of markers near the leptin gene with carcass fat content in Coopworth sheep lines selected for and against fatness

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ABSTRACT

Leptin is an important hormone in controlling food intake and energy expenditure in mammalian species. The present study evaluated levels of serum leptin in Coopworth sheep selected for and against subcutaneous fat depth for 10 generations. Significant differences in serum leptin concentrations were found between fat and lean sheep ($P<0.05$) and between male and female sheep ($P<0.01$). Serum leptin concentration was positively correlated with carcass fatness and there was a clear relationship between mean progeny serum leptin concentrations and the breeding value for fat. Serum leptin concentrations were greater ($P<0.05$) in lambs selected from the fat line (2.69ng/ml) than in lambs selected from the lean line (2.18ng/ml). A microsatellite marker, OarCP26, already mapped on sheep chromosome 4 (OAR4) was located in a sheep yeast artificial chromosome (YAC) clone that was positive for the leptin gene (*LEP*). This confirmed the independent assignment of *LEP,* mapped using a sheep cell hybrid panel, to OAR4. No significant differences in the allele frequencies of OarCP26 and two other flanking markers (TGLA116 and OarHH335) were detected in the lean and fat selection lines suggesting genes other than leptin are causing the differences in fat between the lean and fat Coopworth lines.

Keywords: leptin; sheep; carcass fat content.

INTRODUCTION

The discovery of the obese gene in mice (Zhang *et al.*, 1994) has led to extensive research on its role in obesity in a number of mammalian species. The obese gene codes for leptin, a secreted hormone, which acts to control food intake and energy expenditure. In ruminants, as in other species, leptin is secreted predominately by the adipocytes (Dyer *et al.*, 1997; Ji *et al.*, 1998). Central nervous system administration of leptin to ovariectomised ewes has been shown to inhibit food intake (Henry *et al.*, 1999), and levels of expression of the leptin (*LEP*) mRNA are greater in back, omental and perirenal fat depots in genetically selected fat lines of sheep than in those of the lean lines (Kumar *et al.*, 1998). Serum levels of leptin increase linearly with increased body fat mass and increased energy balance in sheep and cattle (Blache *et al.*, 2000; Ehrhardt *et al.*, 2000). Recently, a missense mutation in the bovine leptin gene has been found to be associated with carcass fat content in cattle (Buchanan *et al.*, 2002). However, the relationship between *LEP* polymorphisms and fat levels is less clear in pigs (Kennes *et al.*, 2001). The aim of the current study was to determine if variants in, or near, the leptin gene were associated with carcass fat content in lean and fat Coopworth sheep lines. The sheep used in the study have been selected for and against liveweight-adjusted ultrasonic backfat depth, over the twelfth rib, since 1981- a period covering 14 years and ten generations (Morris *et al.*, 1997). Thus, they are an ideal model for the study of genes associated with fatness and body composition.

MATERIALS AND METHODS

The experimental work was divided into two sections: 1) measurement of leptin levels in lean and fat sheep lines and 2) statistical analysis of microsatellite markers TGLA116, OarCP26 and OarHH35 in lean and fat sheep lines. Animals used in both sections were generated as part of a long-term selection experiment described by Morris *et al.* (1997).

1) Leptin levels in lean and fat lines

Serum leptin concentrations were measured in 34 lean-line and 36 fat-line animals from the lambs born in 1998. Lambs were the progeny of four sires per line (eight sires). The ram lambs were selected on the basis of their animal model BLUP (best linear unbiased prediction) breeding values for (or against) liveweight-adjusted backfat thickness. The ewes were randomly selected from a pool of available progeny from each sire. Total numbers of lambs analysed ranged from 5-10 per sire.

All animals were ultrasound scanned at six months of age and ultrasonic eye muscle depth B (maximum depth of the *M. longissimus dorsi* over the 12th rib at right angles to the line defining the maximal width) and fat depth C (maximal depth of the *M. longissimus dorsi* over the 12th rib at right angles to the line defining the maximal width and the depth of fat at the point adjacent to measurement B) were measured (Palsson, 1939). GR (grading position) was measured (total tissue thickness perpendicular to the surface measured between the surface of the carcass and the rib 11cm from the vertebral column in the region of the 12th rib) (Kirton *et al.*, 1984) using ultrasound, at the same time.

A serum sample was collected after jugular venipuncture. Serum samples were stored at -18 ℃ until analysis. Serum leptin concentrations were determined,

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in duplicate, using a commercial radioimmunoassay kit ("Multi-Species Leptin RIA Kit", LINCO Research Inc., Missouri, USA). The antibody used in the kit was raised against human leptin, but was reported to display broad cross-reactivity to leptin molecules of many species. Sheep were listed as a recommended species for analysis on the kit documentation. To ensure repeatability of the assay, we ran low and high standards from the kit. These were 3.6 and 29.1 ng/ml respectively and had low coefficients of variation (7.7% and 6.3% respectively).

Selection-line differences in serum leptin concentrations were analysed using the PROC MIXED procedure in SAS (SAS Incorporated, 1992). Sex and selection line were fitted as fixed effects, and the first-order interaction tested. Sire within line was fitted as a random effect. Selection line differences were tested using the sire-within-line degrees of freedom. The selection-line-by-sex interaction term was not statistically significant and was removed from the analysis. The relationship between serum leptin concentration and carcass fatness was assessed using the PROC GLM procedure in SAS (SAS Incorporated, 1992). Leptin concentrations were analysed in a model with ultrasound C measurement as a covariate and sex as a fixed effect. The first-order interaction was found to be non-significant and was, therefore, dropped from the analysis.

2) PEDDRIFT analysis of microsatellites in lean and fat lines

A YAC clone (251C7) containing the *LEP* gene, within a YAC of approximately 200kb, was identified from the AgResearch sheep yeast artificial chromosome (YAC) library (Broom & Hill, 1994). The YAC library was screened using primers designed from the human cDNA sequence (GenBank accession number: hsu18915). The sheep PCR products were sequenced and found to be 90% identical to the human *LEP* sequence. DNA from a panel of sheep-hamster cell hybrids (Burkin *et al.*, 1998) was used as a template for *LEP* primers which amplified a sheep-specific product in the hybrids containing OAR4 (data not shown). The assignment to sheep chromosome four was consistent with its localisation on cattle chromosome four and human chromosome seven (7q32.1). Microsatellite marker OarCP26 was identified within the YAC clone by PCR amplification using the published forward and reverse primers for this microsatellite (Ede *et al.*, 1995). As the sheep YAC clone contained an insert of sheep DNA of about 200kb, this meant the microsatellite OarCP26 and ovine *LEP* were within 200kb of each other. The marker OarCP26 has been localised by linkage analysis at 89cM on OAR4 (de Gortari *et al.*, 1998; Maddox *et al.*, 2001). OarCP26 is flanked proximally by TGLA116 (located between 38 and 66cM) and OarHH35 distally (located at 103 cM) (Maddox *et al.*, 2001).

DNA was extracted (Montgomery and Sise, 1990) from a sample of lean and fat selection line lambs and their sires (30 progeny per line). This group comprised the top and bottom 10% distribution "tails" of both lines when the animals were ranked by fat breeding values. The markers were amplified and scored as described by de Gortari *et al.* (1998). PEDDRIFT (Dodds and McEwan, 1997) was used to analyse whether the frequency of the alleles of the markers TGLA116, OarCP26 and OarHH35 differed in the lean and fat lines.

RESULTS AND DISCUSSION

Both sex and line effects on serum leptin concentration were significant ($P<0.001$ and $P<0.05$, respectively) (Table 1). The fat line animals had significantly higher concentrations than lean line animals and females had significantly higher concentrations than males (Table 1).

TABLE 1: Serum leptin (LS) concentrations and standard error of the mean (s.e.m.) in lean and fat Coopworth selection lines

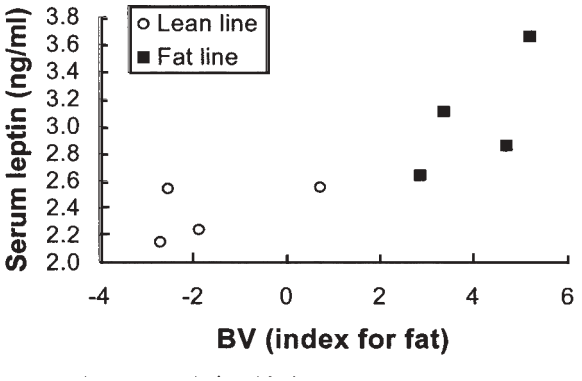
		LS Mean (ng/ml)	s.e.m.
Line			
	Selection lean	2.18	0.17
	Selection fat	2.69	
Sex			
	Ram lamb	2.14	0.14
	Ewe lamb	2.73	

Both carcass fatness (as assessed by fat depth C) and sex had significant effects ($P<0.001$) on serum leptin concentration. Serum leptin concentration was positively related to fat depth C and accounted for 37.6% of the total variation in serum leptin concentration after adjusting for differences due to sex. Every millimetre increase in fat depth C was associated with a 0.15ng/ml increase in serum leptin concentration. Ewe lambs had 0.49ng/ml higher serum leptin concentration than ram lambs at any given fat depth.

There was a clear relationship between mean progeny serum leptin concentrations and the breeding value for fat (Figure 1) in the lean and fat selection lines. Serum leptin concentrations were greater in lambs selected from the fat line than in lambs selected from the lean line. This is in agreement with research carried out on the same lean and fat selection lines by Kumar *et al.* (1998). They showed that leptin mRNA levels were approximately twofold higher in the fat line compared with the lean line in back, omental and perirenal fat depots of ram lambs. Higher levels of circulating leptin in the fat lines than the lean lines are consistent with the increase in fatness seen in the fat lines. Differences in serum leptin levels, in various beef lines have also been found (Geary *et al.*, 2003). These differences were correlated with carcass composition, in particular, marbling score, back fat depth, kidney, pelvic and heart fat and quality grade (Geary *et al.*, 2003).

Chi Square analysis showed there were significant differences ($P<0.01$) in OarCP26 allele frequencies in the lean and fat lines. However when PEDDRIFT (Dodds & McEwan, 1997) was used, to account for any genetic drift that had occurred through selection, there were no significant differences in TGLA116, OarHH35 or OarCP26 allele frequencies between the lean and fat lines. QTL analysis subsequently confirmed this (data not shown). No QTLs were identified for carcass traits in the region of chromosome four associated with leptin. The PEDDRIFT and QTL analyses show that genetic differences in the lean and fat lines are unlikely to be due

FIGURE 1: Relationship between mean progeny serum leptin and the breeding value (BV) for fat in eight rams from lean and fat Coopworth selection lines



to differences in or around the leptin gene. It is likely that the observed differences in leptin concentrations are a secondary consequence of fatness. This lack of association indicates there are other genes causing the differences between the lean and fat Coopworth lines.

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