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Relationships among body composition, circulating concentrations of leptin and follistatin, and the onset of puberty and fertility in young female sheep

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ABSTRACT

The onset of puberty depends on the attainment of critical body mass, so should also be affected by increases in the rate of accumulation of muscle and adipose tissue. Adipose tissue and reproduction are linked by leptin. For muscle, a link has not yet been identified, although one possibility is follistatin. We assessed the relationships among circulating concentrations of follistatin and leptin and the rates of growth and accumulation of muscle and fat during pubertal development in female sheep. We used 326 animals with known phenotypic values for live weight (LW), depths of eye muscle (EMD) and fat (FAT), and known breeding values at post-weaning age for body mass (PWT) and depths of eye muscle (PEMD) and fat (PFAT). Leptin concentration was positively correlated with values for EMD, PEMD, FAT, PFAT, LW and PWT ($P < 0.001$), whereas follistatin concentration was negatively correlated with values for EMD and PWT ($P < 0.001$), and PEMD ($P < 0.01$) and FAT ($P < 0.05$). Leptin concentration was negatively related to age and positively related to live weight at first oestrus and the proportion of females that attained puberty ($P \leq 0.05$), and to fertility and reproductive rate ($P < 0.01$). Follistatin concentration was negatively related to live weight at first oestrus and to fertility ($P < 0.01$) and reproductive rate ($P < 0.05$). There were positive correlations ($P < 0.001$) between muscle accumulation and leptin concentration, and between muscle accumulation and reproductive performance. We conclude that leptin and follistatin are probably both involved in effects of accelerated accumulation of muscle and adipose tissues on the onset of puberty.

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1. Introduction

The onset of puberty depends on an interaction between chronological age and accumulation of body mass so, for example, female sheep usually enter puberty when they attain 50–70% of their mature body mass (Hafez, 1952; Dýrmundsson, 1973). The 'body mass' concept has recently been refined by consideration of individual tissue types and we now appreciate that, in young ewes, genetic merit for accelerated accumulation of muscle and fat is associated with advanced puberty and improved fertility (Rosales Nieto et al., 2013a,b).

The dependency of puberty on tissue mass reveals the importance of physiological signals from metabolic regulatory tissues to the reproductive axis (Martin et al., 2008). For adipose tissue, the primary signal is leptin (review: Foster and Nagatani, 1999) but, for muscle, endocrine factors associated with reproduction have not been clearly identified. One possibility is follistatin, a binding protein that inactivates several members of the transforming growth factor β family, including activin and myostatin, with diverse effects on growth, metabolism, immunity and reproduction (Rodino-Klapac et al., 2009; Hedger et al., 2011). Follistatin is secreted by the sheep ovary (Tisdall et al., 1992) but circulating concentrations vary little during the oestrous cycle (McFarlane et al., 2002), probably because it is produced in a variety of tissues, particularly muscle, where its importance for muscle growth and development has been demonstrated (Matzuk et al., 1995; Lee and McPherron, 2001; Gilson et al., 2009). With respect to reproduction, follistatin seems to have no effect on hypothalamic GnRH secretion in sheep (Padmanabhan et al., 2002), but it does act at pituitary level to inhibit FSH secretion in rodents (Ueno et al., 1987). The ultimate trigger for the first ovulation at puberty might be GnRH and LH pulses, but FSH is essential for the months-long process of ovarian development leading to that point – without it, there would be no follicular development, no oestradiol production and therefore, no ovulation (Schwartz, 1974). Follistatin also appears to play a direct role in ovarian function – in mice, deletion of follistatin in adult granulosa cells leads to effects that range from reduced fertility and litter size to complete termination of ovarian activity and reproduction (Jorgez et al., 2004; Kimura et al., 2010). Overall, therefore, follistatin appears to play roles in oocyte maturation and in the inhibition of pituitary FSH synthesis (Shimasaki et al., 1989; review: Knight and Glister, 2001; Knight et al., 2012).

Thus, we hypothesized that accelerating the onset of puberty and improving reproductive performance by increasing the accumulation of muscle and fat will be associated with changes in the circulating concentrations of leptin and follistatin. We tested this hypothesis in young female sheep, using large field studies in which we analyzed the statistical relationships among leptin and follistatin concentrations, phenotypic and genotypic values for rates of growth and accumulation of muscle and adipose tissues, age and live weight at puberty, fertility and reproductive rate. Large correlation-based studies can detect potential physiological linkages so are valuable as a first step towards the development of hypotheses that guide intervention studies.

2. Materials and methods

These experiments were undertaken in accordance with the Australian Code of Practice for the Care and Use of Animals for Scientific Purposes and were approved by the Animal Ethics Committee of the Department of Agriculture and Food, Western Australia.

2.1. Experiment 1

2.1.1. Experimental location and animals

We used Merino ewe lambs ($n = 136$) that had been born in August–September 2009 on a commercial farm ('Moojepin'). The dams (mothers) of the experimental animals had been sourced from two ram-breeding flocks in Western Australian and sires (fathers) were chosen to supply a wide range in Australian Sheep Breeding Values (ASBV) for growth, muscle and fat. Data were collected for birth date, birth weight, birth type (single or twin) and rear type to weaning (single or twin). Ewes were transported to Medina Research Station (32.2° S, 115.8° E) where the experiment was conducted from February to June 2010. Merino sheep present an extended breading season and the months with lowest ovarian activity are November and December, although there is variation among years due to environmental conditions (Watson, 1953; Radford, 1959). The ewes were weighed bi-weekly and the data were used to calculate the average daily gain (ADG) and to estimate the live weight at puberty and the date of conception. The depths of the *longissimus dorsi* muscle and subcutaneous fat at a point 45 mm from the midline over the twelfth rib were measured using ultrasound when the ewes were 164 (range 134–176) and 251 (range 221–263) days of age. For both measurements, the range in eye muscle depth (EMD) was 20–33 mm and the range in C-site fat depth (FAT) was 2–8 mm. Using MERINOSELECT (Brown et al., 2007), the ultrasound data were used to generate estimates of Australian Sheep Breeding Values at post-weaning age, which can be measured from 7 to 10 months of age, for weight (PWT; range 0–9 kg), depth of eye muscle (PEMD; range 0.0–2.6 mm) and depth of fat (PFAT; range 0.0–1.2 mm). In this year, the national average values in MERINOSELECT for females were 0.9 for PWT, 0.0 mm for PFAT and 0.2 mm for PEMD.

2.1.2. Animal management and feeding

Animals were initially allocated on the basis of live weight to two 20 m × 60 m pens, where they had *ad libitum* access to water and to a pelleted diet (introduced over a 7-day period). The pellets were based on barley, wheat and lupin grains, cereal straw and hay, canola meal, minerals and vitamins, and had been formulated to provide 11.5 MJ of metabolizable energy per kilogram of dry matter, 15% protein, and sufficient minerals and vitamins for maximum growth.

On February 24 (Day −69), when the ewes were 179 days old (range 149–191) and weighed 36.8 ± 0.4 kg, four Merino wethers (male sheep castrated before puberty)