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Cortisol response to ACTH in lambs selected for or against fatness: Effects of maturity and fasting

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ABSTRACT

Although several studies have implicated cortisol in the control of body composition in sheep, the precise mechanisms are unclear. Cortisol basal concentration and response to an ACTH challenge was studied in Coopworth wethers selected for (Fat) or against (Lean) fatness in both the fed and 48 hour fasted states at two ages (15 and 33 weeks). The aim was to investigate whether fasting, age or genotype would influence cortisol secretory parameters.

Mean basal cortisol concentration was higher for fasted than fed lambs (13.6 versus 8.9 ng/ml, $p < 0.001$), but did not differ significantly with genotype or overall stage of development. Maximal cortisol response to ACTH was greater for the fat than the lean genotype, at both 15 weeks (86.3 versus 65.3 ng/ml, $p < 0.01$) and 33 weeks (108.2 versus 91.1 ng/ml, $p < 0.01$) of age. Maximal cortisol response was also significantly greater with fasting and increasing age, but the effect of fasting on cortisol increase was greater at 15 than 33 weeks of age. Clearance of cortisol was significantly slower after fasting, but was unaffected ($p > 0.05$) by genotype and development.

Basal cortisol concentration was not associated with differences in body composition between the fat and lean genotypes. However the larger maximal response in the fat genotype may indicate that the adrenal gland plays a role in determining body composition.

Keywords: Lean; fat; cortisol; adrenal; fasted; fed; development; sheep.

INTRODUCTION

Cortisol is a glucocorticoid secreted from the adrenal cortex. A high basal concentration of cortisol is associated with decreased growth rate and increased fatness, for example, in humans with Cushings syndrome (Dixon *et al.*, 1967). Fatness in different breeds of sheep and cattle has been associated with a high basal cortisol concentration (Kirton *et al.*, 1974; Trenkle and Topel, 1978; Purchas *et al.*, 1980). Adrenalectomy of obese Zucker rats (*fafa*) resulted in decreased body fat but not body protein content (Fletcher, 1986). Conversely, lambs with basal cortisol concentration artificially elevated by cortisol treatment showed no significant difference in body composition (Purchas, 1973a). Acute increases in plasma cortisol as a product of stress have been shown to sensitize adipose tissue for lipolysis (Forse *et al.*, 1987).

Little is known of how cortisol regulates body composition. Basal cortisol is an integration of secretory events under both hormonal (Minton, 1994) and neural controls (Vinson *et al.*, 1994). It is possible that parameters describing the cortisol response to an ACTH challenge might be more appropriate factors than basal concentration in determining the influence of cortisol on body composition.

Coopworth sheep which have been selected for (fat) or against (lean) ultrasonic backfat depth since 1979 (Fennessy *et al.*, 1987) were used for the study. Typically, ultrasonic C and GR in the lean line are 37% and 43% of the fat line respectively. The difference between the lines is equivalent to a response of 4 standard deviations of the original base population variation (J McEwan *pers comm.*). Consequently the selection lines provide a within breed model to test the following hypotheses relating to body composition and cortisol:

1. Basal cortisol concentration will differ between genetically lean and fat animals and with feed status and stage of development.
2. Adrenal response to ACTH challenge will differ between genetically lean and fat animals, and with feed status and stage of development.

MATERIALS AND METHODS

Animals and feeding

20 male lambs were weaned when 10 weeks old and remained on pasture for two weeks, before being brought indoors for adaptation to a concentrate diet (10.7 MJME/kg DM). Animals were penned in groups during the 3 week adaptation period, but during the trial they were penned individually. They were weighed weekly and feed intake was recorded. Lambs were castrated and oestradiol implants inserted to regulate gonadal steroid feedback as described by Suttie *et al.* (1991). Ten animals from each of the fat and lean genotypes were used for the experiment. Lambs were slaughtered at 33 weeks of age. Carcasses were halved and the left side minced for body composition analysis (Lord *et al.*, 1988).

Blood sampling and ACTH challenge

At two developmental stages, 15 weeks and 33 weeks of age, all animals were twice challenged with synthetic ACTH (0.25 mg/ml; Synacthen, Ciba) via a previously inserted jugular cannula, first in the fed state, and subsequently after a 48 hour fast. On each sampling occasion 4 ml of blood was collected via the jugular catheter at 12 minute intervals and stored on ice in heparinized polypropylene tubes until cen-

trifugation. Three baseline samples were collected before ACTH was administered. All lambs were then challenged with ACTH (0.3 µg/kg liveweight) and blood sampling continued for a further 9 samples. Blood was centrifuged at 2000 g for 15 minutes, plasma was removed and stored at -20°C in 5 ml polypropylene tubes.

Cortisol assay

Cortisol concentration was measured using a heterologous competition solid phase assay (Coat-a-count, Diagnostic Products Corporation, Los Angeles, USA). The sensitivity was 0.9 ng/ml using a 50 µl sample. The coefficients of inter- and intra-assay variation for pools containing 66.6, 35.1 and 15.5 ng/ml were 9.2, 10.1 and 16.6 and 4.7, 7.5 and 10.4% respectively. To validate the assay for sheep plasma, samples containing high apparent values for cortisol were diluted in parallel with the standard curve of the assay.

Statistical methods

Fed and fasted cortisol profiles for each animal C(t) for t ≥ 0 (cortisol concentration after the ACTH challenge) was modeled by the equation:

$$C(t) = b + atr^t + e,$$

where t= time (minutes), b=basal cortisol concentration (ng/ml), a=initial dose (ng/ml), r=clearance rate parameter (relating to time in minutes) and e=independently distributed random error. From the equation, the area under the curve and above the baseline (A), the time at which the curve is at a maximum (t_{max}, minutes) and the peak cortisol concentration at t_{max} (peak, ng/ml) were estimated.

Mean pre-challenge cortisol concentration (C_i), mean (C_{mean}) and maximum (C_{max}) post- challenge cortisol concentration, as well as the estimated parameters r, b, a, A, t_{max} and peak for each curve, were analyzed by analysis of variance, with age within animal as the block structure, and categorical factors for, genotype, nutritional regime (fed or fasted), age, and their interactions, as the treatment structure.

RESULTS

Carcass composition

The fat genotype animals contained 38% fat on a dry matter basis compared with 29% for the lean animals

(p<0.01) when corrected for body weight, and lower protein content (13 versus 15%, p<0.01).

Basal cortisol

Mean C_i values were significantly higher for fasted (13.6 ng/ml) than fed (8.9 ng/ml, SED=1.09, p<0.001) animals, but did not differ significantly between fat and lean genotypes.

Cortisol response to ACTH

Area under the curve (A) and cortisol clearance rate (r) did not differ significantly with genotype (Table 1). C_{max} (p<0.01) and peak (p<0.01) values were 24% higher for fat than for lean genotype animals at 15 weeks and 16% higher at 33 weeks of age (Table 1).

Nutritional regime influenced the cortisol response variables C_{max} and C_{mean} (Table 2), with fasted animals having 24.6% larger, more prolonged peaks (3.5 minutes) than fed animals (p<0.001). The difference in the maximal cortisol response (C_{max}) of 15 week old lambs compared to 33 week old lambs in the fed state was 32.7 ng/ml. This is 216% greater than that of fasted lambs, whose difference was only 15.1 ng/ml (SED=5.41, p<0.05). The parameters A, t_{max} and peak, all showed significant (p<0.001) increases with fasting (Table 2) while cortisol clearance rate (r) decreased with fasting (p<0.001).

DISCUSSION

Our results indicate basal plasma cortisol concentration was not different between genotypes. This agrees with Purchas (1973a) who showed that increasing cortisol concentration with cortisol acetate did not alter body composition, but conflicts with the hypothesis that basal cortisol concentration is associated with body composition. Studies which have shown a positive correlation between basal cortisol concentration and body fatness (Kirton *et al.*, 1974; Trenkle and Topel, 1978) used different breeds of sheep for the comparison. In the present study when challenged with ACTH, the maximal cortisol response was larger for the fat genotype at both 15 weeks and 33 weeks of age compared with the lean genotype. This provides support for a concept that adrenal response, rather than basal cortisol concentration, is associated with differences in body composition.

TABLE 1: Effects of genotype on the cortisol challenge parameters basal cortisol (C_i, ng/ml), mean (C_{mean}, ng/ml) and maximum (C_{max}, ng/ml) and the fitted parameters clearance (r), area under the curve (A), time to reach maximal response (t_{max}, minutes) and estimated peak cortisol concentration (peak, ng/ml) at 15 and 33 weeks. Significance levels are denoted by *** = P<.001; ** = P.01; * = P<.05; ns = P>0.05 for the main effects of age (A) and genotype (G) and their interaction (A.G).

	15 weeks		33 weeks		SED1 ¹	SED2 ²	A	G	A.G
	Fat	Lean	Fat	Lean					
Basal (C _i)	11.6	11.2	9.5	12.6	1.60	1.79	ns	ns	ns
Mean (C _{mean})	45.9	36.2	57.4	54.6	3.47	4.49	***	ns	ns
Maximum (C _{max})	86.3	65.3	108.2	91.1	5.95	6.94	***	**	ns
Clearance (r)	0.638	0.642	0.646	0.674	0.017	0.020	ns	ns	ns
Area (A)	507	411	663	617	52.5	59.0	***	ns	ns
Time to max (t _{max})	27.4	28.2	28.0	31.3	1.75	2.14	ns	ns	ns
Peak cortisol (peak)	82.2	61.7	104.3	86.3	5.8	6.3	***	***	ns

¹ for comparisons between ages within genotype

² for comparisons between genotypes at the same age

TABLE 2: Effects of feeding and fasting on the cortisol challenge parameters basal cortisol (C_i , ng/ml), mean (C_{mean} , ng/ml) and maximum (C_{max} , ng/ml) and the fitted parameters clearance (r), area under the curve (A , ng/ml), time to reach maximal response (t_{max} , minutes) and estimated peak cortisol concentration ($peak$, ng/ml) at 15 and 33 weeks. Significance levels are denoted by *** = $P < 0.001$; ** = $P < 0.01$; * = $P < 0.05$; ns = $P > 0.05$ for the main effects of age (A) and nutrition (N) and their interaction (A.N).

	15 weeks		33 weeks		SED ¹	SED ²	A	N	A.N
	Fed	Fast	Fed	Fast					
Basal (C_i)	8.9	14.0	8.9	13.1	1.54	1.57	ns	***	ns
Mean (C_{mean})	29.4	52.7	47.7	64.3	2.67	3.10	***	***	ns
Maximum (C_{max})	59.6	92.0	92.3	107.1	4.80	5.41	***	***	*
Clearance (r)	0.623	0.658	0.644	0.675	0.12	0.14	ns	***	ns
Area (A)	339	579	560	721	37.4	45.6	***	***	ns
Time to max (t_{max})	26.0	29.5	27.8	31.4	1.08	1.44	ns	***	ns
Peak cortisol ($peak$)	57.2	86.7	90.2	100.4	4.7	5.3	***	***	**

¹ for comparisons between ages within nutrition

² for comparisons between nutrition at the same age

The mechanism of increased adrenal sensitivity may be due to the interactions of cortisol with insulin and growth hormone (GH). Insulin promotes growth by stimulating fat and protein synthesis, whereas GH promotes lean growth. Cortisol decreases insulin secretion thereby decreasing the rate of glucose removal as shown by Bassett *et al.* (1967). Krieg *et al.* (1991) have shown that cortisol increases the GH secretory capacity of the pituitary cells *in vitro* but growth studies show that liveweight gain is reduced. This provides evidence that cortisol disrupts the action that insulin and GH have on growth. The altered hormone pattern may direct metabolism towards lipogenesis rather than protein synthesis. Consequently, in the present study, cortisol secretion could influence body composition by altering the pattern of nutrient partitioning.

In agreement with Purchas (1973b), fasting increased basal cortisol concentration. The cortisol response to ACTH of fasted animals was larger than that for the same animals in the fed state. Akana *et al.* (1992) have shown that the adrenal gland of previously stressed animals remains responsive to subsequent stress. In the present experiment, animals were stressed initially by fasting and subsequently with an ACTH challenge. The data show that the adrenal gland is still capable of a large release of cortisol. The difference in the cortisol response to ACTH between the fed and fasted lambs at 15 weeks was significantly greater than that at 33 weeks. This is despite the fact that basal cortisol concentration did not change with age. There are two possible explanations for this result. The first is that there is a maturational change in the adrenal gland such that with an increase in age the adrenal gland is regulated by factors other than ACTH (Vinson *et al.*, 1994). The second possibility is that the adrenal response to ACTH increases with age. In addition, the reduced difference in cortisol response between feeding and fasting in older lambs may be due to the amount of cortisol released in response to the ACTH dose administered being at a maximum. Fulkerson *et al.* (1982) found that cortisol response is increased as ACTH dose increased. Consequently it might be that the 0.3 mg/kg dose delivered to the 33 week old lambs was not enough to initiate the same response as in 15 week old lambs.

The differences in body composition between the fat and lean genotypes are not associated with basal cortisol levels. However the cortisol response to an ACTH challenge

was significantly different between the genotypes, indicating that the adrenal gland may have a role in determining body composition. The mechanisms involved in the developmental effect need to be further investigated.

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