Effects of Age on Fasting-Induced Changes in Insulin, Glucose, Urea Nitrogen, and Free Fatty Acids in Sera of Sheep[1] (43048)

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Abstract. **The hypothesis that prepubertal ewe lambs are metabolically different from postpubertal ewes was tested. Ovariectomized ewes (4 years of age; $n = 4$) and lambs (6 months of age; $n = 4$) were fasted for 72 hr. Serum concentrations of insulin, glucose, urea nitrogen, and free fatty acids (FFA) were measured in blood samples taken at 6-hr intervals between 30 hr before and 72 hr after feed removal. Serum concentrations of urea nitrogen and glucose were not different ($P > 0.20$) between age groups before fasting. Serum concentrations of insulin in ewes increased toward the end of the prefast period whereas those in lambs did not (age × time, $P < 0.01$). Serum concentrations of FFA in ewes tended to be lower ($P < 0.07$) than those in lambs prior to fasting. During fasting, concentrations of insulin decreased ($P < 0.02$) over time in ewes and lambs and did so in a similar manner (age × time, $P > 0.70$). Urea nitrogen increased ($P < 0.0001$) in both fasted ewes and fasted lambs in a comparable manner (age × time, $P > 0.20$). Concentrations of glucose during fasting were not significantly affected ($P > 0.90$) by age. There was a tendency ($P = 0.08$) for concentrations of glucose to change over time but the pattern did not appear to be related to fasting. During fasting, concentrations of FFA tended to be higher ($P < 0.07$) in lambs than in ewes and increased ($P < 0.0001$) in both groups in a similar fashion (age × time, $P > 0.10$). The findings herein suggest that turnover of FFA in lambs may be slightly greater than that in ewes during the fed and fasted states.**
[P.S.E.B.M. 1990, Vol 194]

The critical event leading to onset of puberty in female sheep appears to be an increase in frequency of luteinizing hormone (LH) pulses which stimulates growth of ovarian follicles to the preovulatory stage (1). Increased LH pulse frequency is attributed to decreased responsiveness of the hypothalamic-pituitary axis to estrogen-negative feedback and possibly to a steroid-independent increase in release of gonadotropin-releasing hormone from the hypothalamus (1–3).

The mechanism regulating timing of the prepubertal increase in LH pulse frequency has not been identified. It is likely that this event is influenced by some aspect of growth or metabolism since underfeeding delays onset of puberty as well as the prepubertal decrease in response to estrogen-negative feedback in lambs (3). Steiner *et al.* (4) and Cameron *et al.* (5) proposed that the prepubertal increase in LH pulse frequency is stimulated by blood-borne hormonal and/or metabolic signals which reflect a changing metabolic state. Possible signals which may influence neuroendocrine function include insulin (6–8), glucose (9), free fatty acids (FFA; 10–12), and amino acids (13, 14). Based on this information, we conducted an experiment to determine whether metabolic differences between prepubertal and adult sheep are reflected by differences in serum concentrations of insulin and/or mobilization of energy substrates from muscle and adipose tissue. Since it is difficult to assess metabolic status based on basal concentrations of hormones and metabolites, we evaluated responses of ewe lambs and adult ewes to 72 hr of fasting.

Materials and Methods

Finnish Landrace × Southdown ewes ($n = 4$), which had lambed previously, and prepubertal ewe lambs ($n = 4$), of similar breeding, were ovariectomized

[1] This paper (No. 88-5-177) is published with the approval of the Director of the Kentucky Agricultural Experiment Station.

Received December 8, 1988. [P.S.E.B.M. 1990, Vol 194]
Accepted January 6, 1990.

0037-9727/90/1941-0021$2.00/0

during July 1986 at average ages of 4 years and 4 months, respectively. All sheep were maintained on pasture until August 3, 1986, when they were brought indoors and exposed to a constant ambient temperature (20°C) and artificial photoperiods that simulated natural photoperiods for Lexington, Kentucky (30° 2′ north latitude). Sheep were fed twice daily (0900 and 1500 hr) a complete diet (Table I) at a rate of 50 $g \cdot (kg^{.75})^{-1} \cdot d^{-1}$ and allowed water on an *ad libitum* basis. Two weeks later, animals were subjected to a 72-hr fast. The fast began after the feeding at 0900 hr with time 0 of the fast corresponding to a blood sample drawn at 1200 hr. Blood samples (30 ml) were collected via jugular venipuncture at 6-hr intervals between 30 hr before and 72 hr after feed removal. Average body weights (±SEM) of the ewes and lambs were 62.5 ± 8.1 and 27.0 ± 1.7 kg, respectively.

Assays. All blood samples were placed on ice immediately after collection and allowed to clot overnight at 4°C. Samples were centrifuged at 2620*g* and serum was harvested from each sample and subsequently stored at −20°C until assayed for hormones and metabolites. Serum concentrations were determined for urea nitrogen (UN; 15), glucose (16), and FFA (17) as described previously.

Concentrations of insulin were determined using a radioimmunoassay kit (Micromedic Systems Inc., Horsham, PA). The insulin assay was conducted according to directions provided by the manufacturer. Parallelism was demonstrated by showing that estimates of insulin concentration in a pool of sheep sera were not influenced by sample dilution. Recoveries of 6.2, 3.5, and 1.6 ng of insulin from the serum pool were 109.9, 108.2, and 89.2%, respectively. Assay sensitivity, defined as the amount of insulin at which binding of labeled insulin was 90% of that in assay tubes not containing unlabeled insulin, averaged 0.06 ng/tube. Within- and between-assay coefficients of variation were 5.3 and 11.5%, respectively.

Statistical Analysis. Concentrations of insulin, UN, and glucose in samples collected at 6-hr intervals during both the prefast and fasting periods were subjected to separate analyses of variance for a repeated measures design (18) to determine effects of age, time, and age × time. Mean concentrations of FFA were calculated for samples taken at 18 hr before feed removal and for samples taken at 0, 24, 48, and 72 hr after feed removal. Prefast FFA means were subjected to one-way analysis of variance (18) to determine effects of age. Mean concentrations of FFA during the fasting period were subjected to analysis of variance for a repeated measures design (18) to determine effects of age, time, and age × time. All statistical analyses were done using the Statistical Analysis System (19).

Results

Prefasting Period. There were no significant effects of age on UN ($P > 0.20$) and glucose ($P > 0.90$). In addition, there was no significant effect ($P > 0.20$) of age on insulin. However, one lamb displayed elevated concentrations of insulin as compared with the other three lambs (29.9 ± 3.36 vs 9.12 ± 0.67 ng/ml, respectively). When insulin data from this lamb were deleted from the analysis, a significant ($P < 0.001$) effect of age was apparent as concentrations of insulin were higher in ewes than in lambs. In addition, concentrations of insulin (Fig. 1) in ewes increased toward the end of the prefast period whereas those in lambs did not change (age × time, $P < 0.01$). Concentrations of FFA tended to be higher ($P < 0.07$) in lambs than in ewes (10.49 ± 3.12 vs 4.34 ± 0.70 mg/dl, respectively).

Fasting Period. The effect of age approached ($P =$

Table I. Complete Ration Fed at 50 g/kg Metabolic Body Weight

Ingredient	% Composition
Cottonseed hulls	15.0
Cracked corn	78.0
Soybean meal[a]	5.6
Trace mineralized salt	.5
Ground limestone	.9
Crude protein[b]	10.4

[a] Crude protein = 45%.
[b] Kjeldahl N × 6.25.

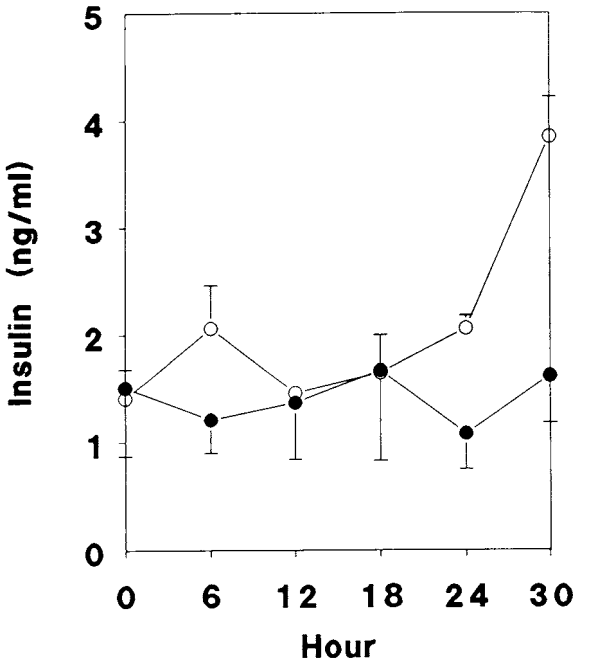

Figure 1. Mean concentrations (±SEM) of insulin in sera of ewes (○) and lambs (●) during the 30-hr prefast period. Each point represents the mean of four animals. Blood samples were taken at 6-hr intervals. Concentrations of insulin increased in ewes but not in lambs (age × time, $P < 0.01$).

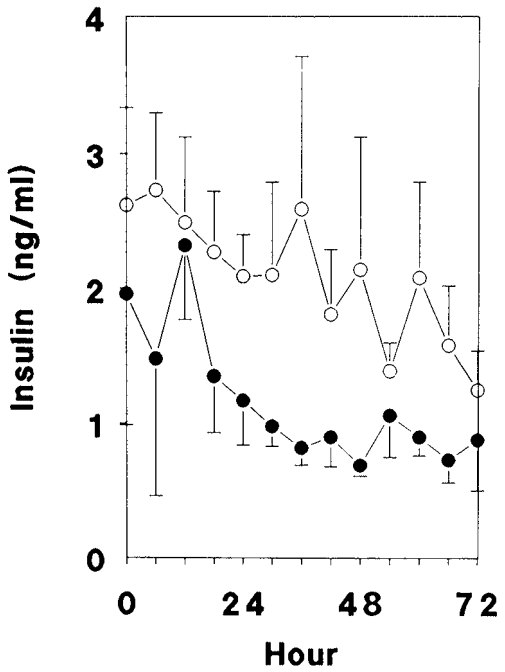

Figure 2. Mean concentrations (±SEM) of insulin in sera of fasted ewes (○) and fasted lambs (●). Each point represents the mean of four animals. Animals were fasted for 72 hr and blood samples were taken at 6-hr intervals. Concentrations of insulin decreased ($P < 0.07$) in both fasted groups in a similar manner (age × time, $P > 0.70$).

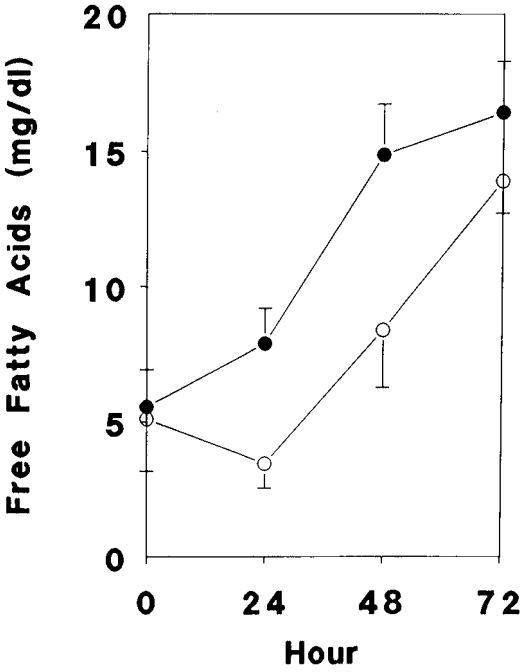

Figure 4. Mean concentrations (±SEM) of free fatty acids in sera of fasted ewes (○) and fasted lambs (●). Animals were fasted for 72 hr and blood samples taken at 6-hr intervals. Each point represents the mean of four animals. Concentrations of free fatty acids tended to be higher ($P < 0.07$) in lambs than in ewes and increased ($P < 0.01$) in a similar manner (age × time, $P > 0.10$).

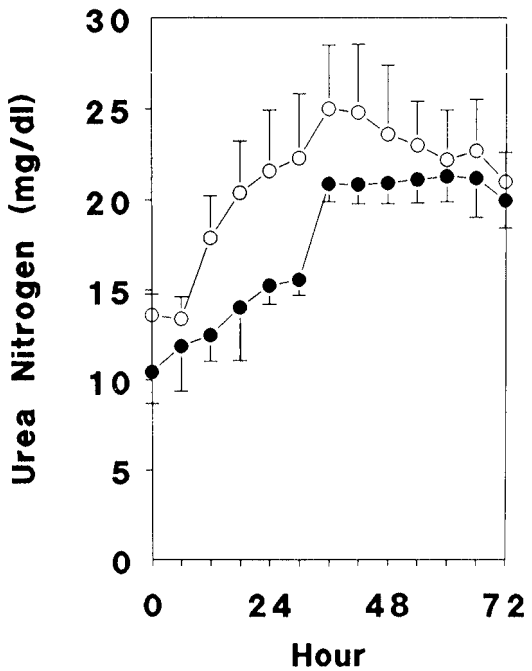

Figure 3. Mean concentrations (±SEM) of urea nitrogen in sera of fasted ewes (○) and fasted lambs (●). Animals were fasted for 72 hr and blood samples taken at 6-hr intervals. Each point represents the mean of four animals. Concentrations of urea nitrogen increased ($P < 0.0001$) in fasted lambs and ewes in a similar manner (age × time, $P > 0.20$).

0.11) significance as concentrations of insulin (Fig. 2) in lambs were lower than those in ewes at all time points. Concentrations of insulin decreased ($P < 0.02$) in both groups in a similar manner (age × time, $P > 0.70$).

Concentrations of UN (Fig. 3) were similar ($P > 0.20$) for ewes and lambs. Concentrations of UN increased ($P < 0.0001$) to approximately twice initial concentrations in both ewes and lambs in a comparable fashion (age × time, $P > 0.20$).

Concentrations of FFA (Fig. 4) tended to be higher ($P < 0.07$) in lambs than in ewes. Mean FFA concentrations increased ($P < 0.0001$) in both fasted groups in a similar manner (age × time, $P > 0.10$). Concentrations of glucose were similar ($P > 0.80$) for ewes and lambs. Although there tended ($P = 0.08$) to be an effect of time, the change in glucose concentrations was not related to fasting. Mean concentrations for ewes and lambs were 61.25 ± 2.04 and 58.94 ± 2.88 mg/dl, respectively.

Discussion

Prefasting Period. Concentrations of insulin in our study were similar between ewes and lambs. This is in contrast to other studies in humans (20) and monkeys (4), which have shown that concentrations of insulin were higher in postpubertal individuals than in prepubertal individuals. In addition, Verde and Trenkle (21) found that concentrations of insulin in growing

steers increased with age. The reason why there were no age-related differences in insulin concentrations in our study may be due to the fact that one lamb had elevated insulin levels in comparison to other lambs in the group. When data from this lamb were removed from analysis, an age effect was apparent. Alternatively, it may be that, at 6 months of age, lambs have matured to the point where basal concentrations of insulin are not greatly different than those in ewes. Concentrations of insulin in our study increased toward the end of the prefast period in ewes but not in lambs. The reason why this occurred is not readily apparent.

Concentrations of FFA tended to be higher in lambs than in ewes. This may be attributed to the fact that animals exhibit increased fat deposition (22) and decreased lipolysis as they mature (23).

Fasting Period. During fasting, concentrations of insulin decreased in both groups. A decrease in insulin concentrations during fasting agrees with previous work in sheep (24), cattle (25), subhuman primates (5), and humans (26). In primates, however, concentrations of insulin during fasting decreased more rapidly and to a lower level in prepubertal individuals as compared with postpubertal individuals. This was not observed in our study. However, it should be noted that concentrations of insulin in lambs were lower than those in ewes at all time points measured. As mentioned previously, it may be that, at 6 months of age, lambs have matured to the point where their response to fasting is not greatly different from that of ewes with respect to insulin concentrations.

Concentrations of UN increased in fasted ewes and fasted lambs. Data concerning changes in concentrations of UN during fasting in ruminants are equivocal. Rule *et al.* (25) showed that concentrations of UN increased in steers during fasting, but Trenkle (27) noted that concentrations of UN in fasted wethers were not different from those in fed controls. Finally, Koenig and Boling (28) found that fasting caused a decrease in concentrations of UN in both 1-year-old and 8-year-old ewes. The reasons for these differences are not readily apparent, but could be related to differences in age, diet, body condition, and/or length of time fasted.

The increase in UN over time due to fasting occurred in a similar manner for ewes and lambs, suggesting that ewes and lambs metabolized amino acids in a similar manner. However, we did not characterize blood amino acid profiles or determine turnover rates of amino acids by various tissues. It is likely that different amino acids were mobilized in lambs than in ewes since Koenig and Boling (28) observed differences in plasma concentrations of specific amino acids between fasted ewes (8 years of age) and fasted yearling lambs. This has also been shown in subhuman primates (5). In addition, it is possible that turnover of certain amino acids in tissues such as the brain differed between young and old sheep.

Concentrations of FFA increased in both fasted ewes and fasted lambs. It is well established that FFA in ruminants increase during fasting (25, 29–31). However, we are the first to report that the fasting-induced increase in FFA was of greater magnitude in lambs than in ewes. In monkeys (5) and humans (20, 26, 32) concentrations of FFA and ketones during fasting were inversely related to age. It appears that as an animal matures, it is less able to mobilize fat from adipose tissue. The difference in FFA mobilization between fasted lambs and ewes may be attributed to differences in endocrine status. In our study, insulin concentrations were not significantly different between fasted lambs and ewes. However, insulin concentrations were numerically lower in lambs than in ewes during fasting. Thus, the lipogenic stimulus supplied by insulin may have been lower in lambs than in ewes. Additional factors such as somatotropin (21, 33, 34) and circulating catecholamines (35–37) may also play a role in bringing about fasting-induced differences in FFA concentrations between pre- and postpubertal individuals.

Concentrations of glucose were not affected by age or fasting. This disagrees with the results of Rule *et al.* (25) and Baird *et al.* (29), who observed that concentrations of glucose decreased by 2 days of fasting in cattle. However, the study by Rule *et al.* (25) suggests that the decrease in glucose concentrations was transient and was followed by an increase on Days 2 to 5 of the fasting period. Carstairs *et al.* (38) did not detect changes in concentrations of glucose in lactating dairy cattle fed a restricted energy diet for an 84-day period.

Concentrations of insulin, UN, and glucose were not different between lambs and ewes prior to or during fasting. Concentrations of FFA were slightly higher in lambs than in ewes prior to fasting. Concentrations of FFA also increased in both fasted lambs and ewes, but increased to a higher level in fasted lambs. This may suggest that turnover of FFA in lambs is greater than that in ewes during fed and fasted states.

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