Glucagon Responses to Exercise in Sheep

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Abstract
Sheep were subjected to moderate (5 km/h) and strenuous (7 km/h) exercise on a treadmill for 45 min. After training, the sheep were again exercised. Glucagon concentrations in plasma increased in all sheep after commencement of exercise. These increases were related directly to the severity of exercise. The glucagon response also was dependent upon training with a lesser increase in trained animals than in untrained animals running at the same speed. Insulin concentrations in plasma decreased significantly only during strenuous exercise in untrained sheep.

[Other keywords: glucose, lactate.]

Introduction
The role of the endocrine pancreas in adaptation to exercise has been explored recently. Whereas results of studies in rats (Luyckx and Lefebvre 1974) and dogs (Bottger et al. 1972) show that glucagon concentrations increase during exercise, the results of studies in man are less convincing. Some investigators report only a modest elevation of glucagon concentrations during exercise (Felig et al. 1972; Galbo et al. 1975), whereas others report no change (Nilsson et al. 1975).

No studies on the effect of exercise on the hormones of the pancreas of ruminant species have been reported. The present study examines the glucagon and insulin responses to moderate and strenuous exercise in sheep.

Materials and Methods
Five adult ewes of mixed breeds, aged 1–2 years and weighing 45–55 kg, were used in this study. They received 400 g of alfalfa pellets twice daily. Water and salt licks were provided ad libitum.

The sheep were exercised on a treadmill (Model 18-72D, Quinton Instruments, Seattle, Washington, U.S.A.). Each sheep was subjected to each of four treatments which included two intensities of exercise. In these experiments the sheep ran for 45 min. Moderate exercise was 5 km/h with the treadmill set at 5% incline. Strenuous exercise consisted of running at 7 km/h with a 10% incline. The first set of experiments was moderate exercise in untrained sheep. About 5 days later the sheep were subjected to strenuous exercise. After training (which consisted of running for about 1 h at 7 km/h on the treadmill on at least six occasions over a 3-week interval) the sheep were subjected to moderate and strenuous exercise again.

Feed was withheld for 24 h before experimentation. At least 12 h before the experiments, polyvinyl catheters (1-00 by 1-50 mm, Dural Plastics, Dural, Australia) were inserted into the jugular veins. The sheep were put on the stationary treadmill and after an adjustment period of 2 h, three control samples were taken from the jugular catheter at 30-min intervals. Exercise was then initiated. Blood samples were taken at 15, 30 and 45 min of exercise. Additional samples were taken 15 and 45 min after termination of exercise.
The blood samples were taken in heparinized syringes and immediately placed into tubes chilled on ice. An aliquot of 5 ml of blood was used to prepare neutral filtrates for metabolite analyses. The remainder of blood was centrifuged immediately at 2°C and the plasma harvested. Plasma for glucagon analyses was stored in tubes containing 500 KIU* Trasylol [Boehringer–Ingelheim (Canada) Ltd, Dorval, Quebec] per ml of plasma. Plasma and filtrates were stored at −20°C until analysed.

Glucose concentrations were determined by the glucose oxidase method (Glucostat, Worthington Biochemical Corp., Freehold, N.J.). Lactate was determined by fluorimetric enzymic assay with lactate dehydrogenase (Olsen 1971) using reagents from Sigma Chemical Co. (St Louis, Missouri, U.S.A.).

Glucagon and insulin were determined by radioimmunoassay using the double antibody technique (Morgan and Lazarow 1963). Antisera to the hormones were developed in guinea pigs. Glucagon antiserum was Manns’ GP26 which is specific for pancreatic glucagon, giving values similar to Unger’s glucagon antiserum 30K (Brockman et al. 1976). The incubation buffer was a 0·05 M phosphate buffer, pH 7·0, containing 1 g gelatin, 1 g EDTA, 200 mg mepihiolate and 8·5 g NaCl per litre of buffer. In the glucagon assay the buffer also contained 250 KIU Trasylol per ml. Standards for both assays were made up in peptide-free ovine plasma. The latter was prepared by stirring 10 g of activated charcoal in 100 ml of plasma for 4 h. The charcoal was then separated from the plasma by centrifugation and filtration. For the glucagon assay, peptide-free plasma was prepared similarly for each sheep and analysed for glucagon with the plasma samples of that sheep. The value obtained for the assay of the peptide-free plasma was then subtracted from the immunoreactive glucagon values of the plasma samples. The sensitivity of the insulin assay was 1 mU/l. Repeatibility within an assay was 5·8 ± 1·1% (n = 12) and repeatability of the same sample on several assays was 6·9 ± 1·4% (n = 24). The sensitivity of the glucagon assay was 20 ng/l and intra- and interassay repeatabilities were 7 ± 2% (n = 10) and 16 ± 3% (n = 11) respectively.

Statistical analyses for point by point tests of significance were t-tests. Each point during and after exercise was tested against the three pre-exercise values with paired t-tests. However, when more general hypotheses, e.g. effect of training, were tested significant differences were determined by analysis of variance and individual degree-of-freedom tests. Since all sheep were subjected to each of the exercise treatments, for most tests of significance paired analyses (randomized block design) were used.

**Results**

The effects of exercise on blood lactate concentrations are presented in Fig. 1a. In all groups lactate concentrations were significantly greater (P < 0·01, paired t-test) during exercise than during the pre-exercise period and remained so for 45 min after exercise, except in moderately exercised trained sheep. It appeared that the concentrations of lactate were useful indicators of the severity of exercise. Blood lactate concentrations were elevated most during strenuous exercise when the animals were untrained. Lactate concentrations during both moderate and strenuous exercise in trained animals were significantly lower (P < 0·05, individual degree of freedom) than during corresponding exercise in untrained animals. Furthermore, in the strenuously exercised animals, lactate concentrations remained significantly greater (P < 0·05) during the postexercise period in untrained animals than in trained animals. Concentrations of lactate during strenuous exercise for trained sheep were the same as those for untrained sheep that were moderately exercised.

Changes in concentrations of blood glucose induced by exercise paralleled the changes for lactate (Fig. 1b). The greatest increase in levels of glucose occurred during strenuous exercise in untrained sheep. During moderate exercise in trained sheep there was no significant change in glucose concentrations from pre-exercise values. The hyperglycaemic response to exercise was significantly reduced (P < 0·01,

*Kallikrein inactivating units.*
during exercise in sheep. Glucose concentrations were also significantly less ($P<0.01$) during moderate exercise than during strenuous exercise.

**Fig. 1.** (a) Blood lactate and (b) blood glucose concentrations (means ± s.e., $n = 5$) before, during and after 45 min of moderate exercise in trained (△) and untrained (●) sheep, and strenuous exercise in trained (▼) and untrained (■) sheep. Open symbols represent values differing significantly ($P<0.05$, paired t-test) from corresponding pre-exercise values. In (a) asterisks denote values which differ significantly ($P<0.05$) for corresponding trained and untrained sheep. In (b) significant differences ($P<0.05$) between values for trained and untrained sheep given strenuous (**) and moderate (*) exercise, respectively, are denoted. Dashed vertical lines indicate the period of exercise.

**Fig. 2.** (a) Plasma glucagon and (b) plasma insulin concentrations (means ± s.e., $n = 5$) before, during and after 45 min of moderate exercise in trained (△) and untrained (●) sheep, and strenuous exercise in trained (▼) and untrained (■) sheep. Open symbols represent values differing significantly ($P<0.05$, paired $t$-test) from corresponding pre-exercise values. Asterisks denote values which differ significantly ($P<0.05$) for corresponding trained and untrained sheep. Dashed vertical lines indicate the period of exercise.
Glucagon concentrations increased significantly \((P<0.05)\) during exercise (Fig. 2a). The greatest increase in level of glucagon occurred during strenuous exercise in untrained sheep. The least response occurred during moderate exercise in trained sheep. The glucagon responses obtained during strenuous exercise in trained sheep and moderate exercise in untrained sheep were intermediate. Changes in concentrations of glucagon were significantly lower for trained than for untrained sheep and during moderate than during strenuous exercise \((P<0.01,\) individual degree of freedom).

The insulin responses to exercise were not consistent (Fig. 2b). The major observation was that during strenuous exercise in untrained sheep insulin concentrations decreased significantly \((P<0.05)\). However, during the other exercise regimes there were no significant changes \((P<0.05)\).

**Discussion**

The blood lactate concentrations were used as an index of anaerobic metabolism (Friedman and Barborka 1941) during exercise to verify that the trained sheep differed from the untrained sheep (Cobb and Johnson 1963). The data (Fig. 1a) show that during exercise in untrained sheep, levels of lactate in blood were markedly higher than during exercise in trained sheep. The lactate concentrations were the same for untrained and trained sheep given moderate and strenuous exercise respectively. The similarity between these groups was also borne out by the concentrations of glucose in blood (Fig. 1b) and glucagon in plasma (Fig. 2a).

Both moderate and strenuous exercise were associated with increases in the levels of glucagon in plasma. Exhaustive prolonged exercise seems to be necessary to enhance glucagon secretion in dogs (Bottger et al. 1972; Vranic et al. 1976), rats (Luyckx and Lefebvre 1974) and man (Felig et al. 1972; Galbo et al. 1975). However, in sheep moderate exercise was a sufficient stimulus to raise plasma levels of glucagon. This glucagon response was even greater with strenuous exercise. In both cases training was associated with a reduced response.

This training-induced lowering of plasma glucagon responses has been reported previously in man (Gyntelberg et al. 1977) and rats (Galbo et al. 1977). Furthermore, in the trained rats the exercise-induced glucagon secretion was eliminated. Nevertheless, the hyperglycaemia was the same as in the untrained rats.

Glucagon is a gluconeogenic and glycogenolytic agent in sheep (Brockman and Bergman 1975) as in non-ruminant animals. Its secretion is depressed by glucose (Brockman 1977) and increased by hypoglycaemia \(in vivo\) (Bloom et al. 1974; Brockman 1977). Catecholamines, also, are known to stimulate glucagon secretion in sheep (Phillips et al. 1969; Bassett 1972). However, the glucagon response during exercise does not appear to be accounted for entirely by catecholamines (Galbo et al. 1975, 1976). Studies in calves in which hypoglycaemia was induced with insulin have implicated the autonomic nervous system in regulating glucagon secretion (Bloom et al. 1973, 1974). Glucagon appears to play a critical role in the sympathetic response of ruminant animals when there are increased demands for glucose.

The decrease in insulin during strenuous exercise is interesting. It is probably due to inhibition of adrenergic receptors of \(\beta\)-cell by catecholamines (Bassett 1970) in order to optimize the mobilization of energetic substrates such as glucose and free fatty acids (Leclercq-Meyer and Malaisse 1975).
In conclusion, the results of the present study showed that exercise is a potent stimulus for glucagon secretion in sheep. Furthermore, the magnitude of the response is dependent upon intensity of exercise as well as conditioning of the animal. This glucagon response may account for the increased gluconeogenesis and glycogenolysis observed in exercising sheep (Jarrett et al. 1976; Judson et al. 1976).

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References


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