# Local Intra-arterial Infusion of Growth Hormone into the Mammary Glands of Sheep and Goats: Effects on Milk Yield and Composition, Plasma Hormones and Metabolites

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*Abstract*

Lactating goats and sheep were fitted with catheters in the external pudendal arteries supplying both mammary glands. Saline was infused continuously into one artery whereas the other artery received continuous infusions, over successive 4-day periods, of either saline or growth hormone (GH) — doses increasing twofold between successive periods from 100 to 400 $\mu$g/day in goats and 400 to 3200 $\mu$g/day in sheep.

Local infusion of GH at up to 1600 $\mu$g/day in sheep did not affect milk yield or composition nor peripheral plasma concentrations of GH, insulin, glucose, urea and non-esterified fatty acids (NEFA). Infusion of GH at 3200 $\mu$g/day in sheep increased peripheral plasma concentrations of GH, tended to increase milk yield and peripheral plasma NEFA but there were no changes in peripheral plasma insulin, glucose and urea. It is concluded that GH does not exert direct effects on the mammary glands of sheep and goats in situations where the hormone is administered over short periods.

## Introduction

Although it is well documented that growth hormone (GH) increases milk production in cows (Bauman *et al.* 1980; Bines *et al.* 1980; Peel *et al.* 1981, 1982), sheep (Dracy and Jordan 1954; Jordan and Shaffhausen 1954; Hart *et al.* 1985) and goats (Hart *et al.* 1980; Mepham *et al.* 1984), the mechanisms by which the hormone exerts galactopoietic effects remain unclear. Bines *et al.* (1980) and also Bauman *et al.* (1980) raised the possibilities that GH acts directly or indirectly on the mammary gland or, alternatively, influences the supplies of key nutrients to the mammary gland, thereby influencing the capacity of the mammary gland to synthesize milk.

The present studies were conducted to establish whether GH exerts direct effects on the mammary glands of lactating sheep and goats. Preliminary results of studies in lactating sheep were reported by McDowell and Hart (1984).

## Materials and Methods

*Experimental Animals*

The three primiparous British Saanen goats and five primiparous English Halfbred ewes were free from obvious abnormalities of the mammary glands. Goats were housed in small pens whereas the sheep were kept in metabolism cages. Both sheep and goats were fed a diet containing 60 : 40 (w : w) sheep pellets (BOCM): chopped pasture hay in sufficient quantities to meet calculated requirements for

metabolizable energy (Anon. 1975). The daily allocation was offered in approximately equal portions at milking times (0830 and 1600 h each day) and water was available *ad libitum*.

All animals were accustomed to handling and could be bled (from jugular catheters) and milked without restraint. As the sheep had not been milked previously, 1 i.u. oxytocin (Sandoz, Basel, Switzerland) was administered intravenously via the jugular catheter immediately prior to each milking throughout the experiment.

Studies with the goats were conducted during summer (June/July) when they had been lactating for 10–14 weeks. The studies on sheep were conducted during winter (December–March) and commenced about 10 days after lambing.

### Surgical Preparation of Animals

Polyvinyl chloride catheters (0·86 mm i.d. by 1·27 mm o.d.; Dural Plastics Ltd, Dural, N.S.W. 2158, Australia) were inserted in both external pudendal arteries of each goat and sheep under halothane anaesthesia. The catheters were tunnelled beneath the skin to exit points high on the back and posterior to the rib cage. An additional polyvinyl chloride catheter (1·0 mm i.d. by 1·5 mm o.d.; Dural Plastics Ltd) was inserted in an external jugular vein of each animal while the animal was anaesthetized. Catheters were kept patent by flushing with minimum amounts of heparinized saline ($2 \times 10^5$ i.u. heparin and 9·0 g NaCl per litre).

All animals recovered from surgery within 5–7 days by which time feed intakes and milk yields had returned to the levels before surgery. The animals remained in good health throughout the experiments.

### Analytical Procedures

#### Milk constituents

Milk fat, protein and lactose were measured using a Milko-Scan 203 (A/S N Foss Electric, Denmark) after appropriate calibration of the instrument with sheep or goat milk.

#### Plasma hormones

Insulin and GH were measured using the radioimmunoassays described by Tindal *et al.* (1978). Concentrations of insulin and GH were expressed relative to the standards bovine insulin (24 i.u./mg) and bovine GH (NIH-GH-B2; 1·5 U/mg) respectively. The sensitivities of the assays were 0·4 mU/l and 0·2 $\mu$g/l for insulin and GH respectively. Assays were considered valid if the intra-assay coefficient of variation was less than 15%. To reduce variation stemming from differences between assays, all samples from a single experiment were measured in the same assay.

#### Plasma metabolites

Glucose and urea were assayed simultaneously in untreated plasma samples using a dual channel ChemLab Autoanalyser as outlined by the manufacturer (ChemLab Instruments Ltd, Hornchurch, Essex, U.K.). Plasma non-esterified fatty acids (NEFA) were assayed by the microtitration procedure described by Kelley (1965).

### Infusates

Saline (9 g NaCl/l) was sterilized by autoclaving. Appropriate quantities of bovine GH were dissolved in sterile saline adjusted to pH 10 with 1 M NaOH. Benzylpenicillin (Glaxo Laboratories Ltd, Greenford, U.K.; 1·67 $\times$ 10$^3$ U/mg) was added to infusates at the rate of 1 mg/ml, and infusates were dispensed into sterile reservoirs with capacity to contain sufficient infusate for 24 h. Bacteriological analyses confirmed the absence of bacteria from infusates.

### Bovine Growth Hormone

Different preparations of bovine GH were used. In the experiment with goats NIH-GH-B18 (0·81 U/mg) was used whereas in the experiment with sheep a preparation extracted on site (ICH/ bGH/5; 1·2 U/mg) was used. The preparations elicited similar galactopoietic effects in sheep and goats when administered subcutaneously at a dose rate of 0·081 U/kg live weight. At this dose milk yields of both sheep and goats increased by 10–15%.

*Experimental Procedures*

Prior to surgery, the sheep and goats were trained to wear harnesses onto which were attached pouches containing infusate reservoirs and a battery-operated peristaltic pump (SIRO pump; Everest Electronics, Seaford, South Australia). When the animals had recovered from surgery, the experiment commenced.

Saline was infused continuously into one external pudendal artery for the duration of the experiment. The other artery received continuous infusions, over alternate periods of 4 days, of either saline or GH. Throughout the experiments, daily doses of GH increased twofold between successive GH infusion periods. Daily doses for goats were 100, 200 and 400 $\mu$g/day whereas doses for sheep were 400, 800, 1600 and 3200 $\mu$g/day. Infusion rates of 5 ml/h were maintained for saline and the GH infusates.

At the conclusion of the infusions to the sheep the galactopoietic potency of the preparation of GH was assessed. Immediately after the last period of saline infusions, each ewe was given a daily subcutaneous injection of 0·1 mg/kg liveweight GH for 4 days, then for a further 4 days a daily subcutaneous injection of saline (pH 10·0). Milk yield and composition, together with plasma concentrations of metabolites and hormones were measured during each of these periods of 4 days.

Goats were milked by hand whereas sheep were milked by machine. Milk from each udder half of each animal was collected separately, pooled over 24 h, weighed and a subsample kept at 4°C after addition of formalin (100 g/l; 3 drops/10 ml milk) as preservative, pending subsequent weekly analyses.

Blood samples were collected from the jugular catheters on day 2 or 3 of each period of the experiments each hour between 1000 and 1500 h. Heparin was used as anticoagulant and plasma separated at 4°C within 10 min of collection was stored at −20°C pending analyses for glucose, urea, NEFA, GH and insulin.

*Statistical Analyses*

Comparisons between values for the parameters measured during individual periods (4 days) of each infusion experiment were analysed by analysis of variance treating the experiments as split plots in time. Differences between mean values for parameters measured during the injection of GH or saline were assessed by paired *t*-tests.
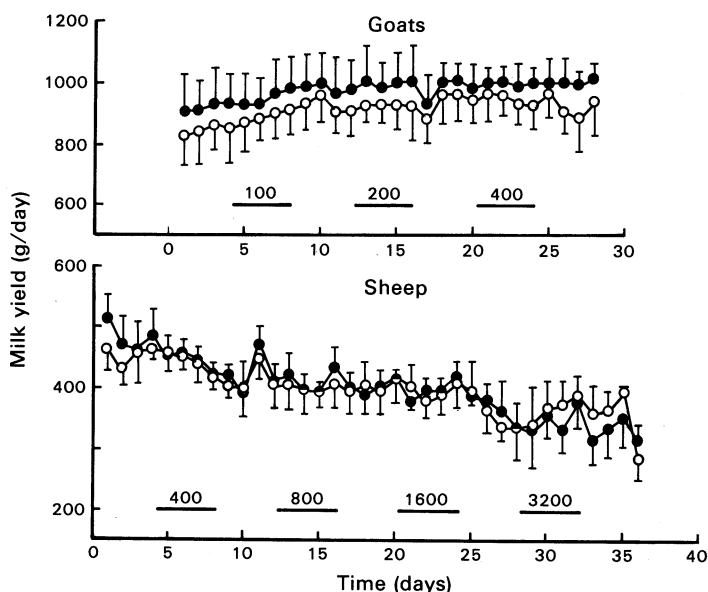


**Fig. 1.** Daily milk yields of mammary glands given continuous intra-arterial infusions of saline (○) or alternating infusions of saline or growth hormone (●) at the daily dose ($\mu$g/day) indicated above horizontal bars. Plotted points represent mean values for three goats and five sheep. Standard errors are shown as vertical bars.

## Results

### Milk Yield and Composition

Mean milk yields during infusions are shown in Fig. 1. Although there were significant trends ($P < 0.05$) over the period of the experiment (yields of goats increased and those of sheep decreased), there were no significant differences ($P > 0.05$) between yields of glands given continuous intra-arterial infusions of saline (control glands) and those given alternating intra-arterial infusions of saline and GH (treated glands). It appeared that the yields of both control and treated glands of the sheep increased during infusion of GH of 3200 μg/day but these changes were not significant ($P > 0.05$).

During injection of sheep with GH, milk yield increased significantly ($P < 0.05$) from 644 ± 41·7 g/day (mean ± s.e.m.) during the last period of saline infusion to 724 ± 27·7 g/day. Yield then decreased significantly ($P < 0.05$) to 659 ± 28·2 g/day during the 4 days when saline was injected.

No significant ($P > 0.05$) effects of GH infusions on milk composition were measured in sheep and goats. However, there were changes in composition of milk from both treated and control glands throughout the experiments. In the goats, there were significant changes over the period of the experiment ($P < 0.05$) in contents of milk fat and protein in both control and treated glands. Contents of milk fat decreased from *c.* 42 to 38 g/kg and milk protein decreased from *c.* 31 to 29 g/kg over the 28 days of the experiment. Milk lactose content remained essentially constant at *c.* 45·5 g/kg.

Similar trends for changes in milk composition were recorded for the sheep. Milk fat content, although very variable, decreased significantly ($P < 0.001$) from *c.* 80 to 70 g/kg and milk lactose decreased significantly ($P < 0.001$) from *c.* 47 to 44 g/kg throughout the experiment. No significant ($P > 0.05$) changes were measured for milk protein content which remained at *c.* 60 g/kg.

In the sheep injections of GH did not affect lactose and protein contents of milk which remained at *c.* 44 g/kg and 60 g/kg respectively. There was, however, a significant effect ($P < 0.05$) of GH injection on milk fat. Values were 70·3 ± 3·62 g/kg (saline infusion) 77·9 ± 2·62 g/kg (GH injection) and 71·4 ± 2·18 g/kg (saline injection) during the respective periods of 4 days.

### Plasma Hormones

#### Growth hormone

Mean daily values, on the second or third day of each period of infusions are shown in Fig. 2. In the goats, there were no significant differences ($P > 0.1$) between values for plasma GH in individual periods. However, in general, values were lower ($P < 0.1$) during GH infusion than control periods. For the sheep, plasma concentrations of GH were significantly increased ($P < 0.001$) during infusion of GH of 3200 μg/day and also during the last control period. Values for all other periods were similar (*c.* 2·5 μg/l).

Plasma GH increased significantly ($P < 0.05$) during injections of GH in the sheep. Values were 5·2 ± 0·74 μg/l (saline infusion), 11·0 ± 0·77 μg/l (GH injection) and 3·3 ± 1·08 μg/l (saline injection) respectively.
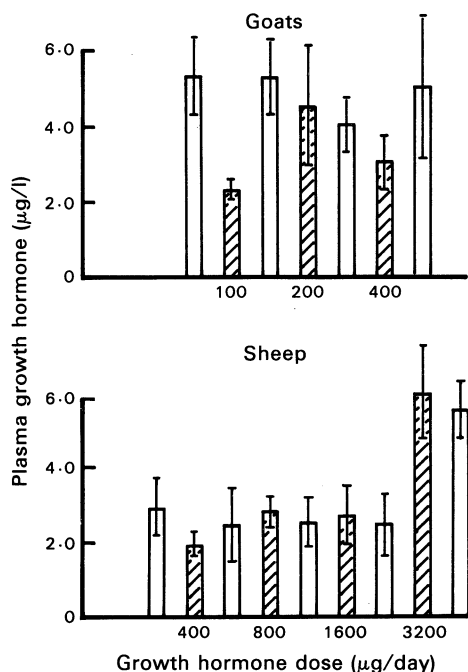
**Fig. 2.** Concentrations of plasma growth hormone for three goats and five sheep given continuous infusions of saline (open histograms) or growth hormone (hatched histograms) at the daily dose indicated, into one mammary artery. Histograms represent mean values for the second or third day of respective infusion periods of 4 days. Standard errors are shown as vertical bars.

*Insulin*

In both goats and sheep plasma insulin concentrations remained relatively stable (goats 22–27 mU/l; sheep 14–20 mU/l) throughout the experiments. There were no effects of GH infusions in either sheep or goats. Similarly injections of GH did not affect plasma insulin which remained at $c.$ 20 mU/l.
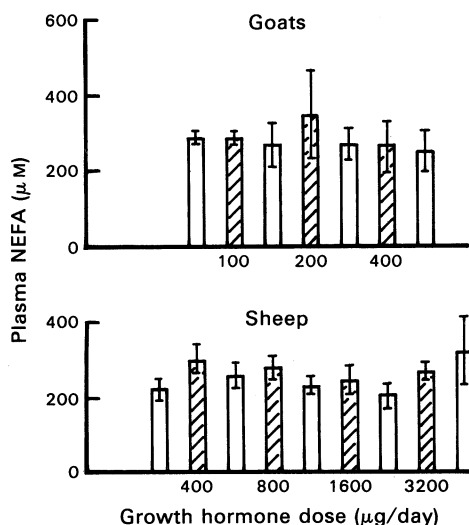


**Fig. 3.** Concentrations of plasma NEFA for three goats and five sheep given continuous infusions of saline (open histograms) or growth hormone (hatched histograms) at the daily dose indicated, into one mammary artery. Histograms represent mean values for the second or third day of respective infusion periods of 4 days. Standard errors are shown as vertical bars.

*Plasma Metabolites*
  *Glucose and urea*

Growth hormone infusions had no effects $(P > 0.1)$ on plasma concentrations of glucose and urea. Mean daily concentrations of glucose were $c.$ 3·5 and

*c.* 4·0 mM for goats and sheep respectively. Corresponding values for plasma urea were *c.* 5·1 and 5·0 mM respectively. There were no effects of GH injections or plasma glucose and urea in the sheep. Concentration of glucose remained at *c.* 4 mM and urea *c.* 5 mM.

### Non-esterified Fatty Acids

Mean concentrations of plasma NEFA on the second or third day of each period of respective infusions are shown in Fig. 3. There were no significant effects ($P > 0·1$) of GH infusions on plasma concentrations of NEFA in the goats. Similarly, in the sheep differences between values for respective periods were not significant ($P > 0·1$). In spite of the latter, mean daily concentrations tended to be higher during GH than saline infusions. Indeed, for the sheep comparison of values for control and GH-infusion periods, combined for all doses of GH, indicated a significant ($P < 0·1$) effect of GH infusion.

Plasma NEFA were similar in the sheep during the last saline infusion period ($323 \pm 89$ $\mu$M) and during injection of GH ($319 \pm 111$ $\mu$M), and although the mean value during injection of saline ($232 \pm 17$ $\mu$M) was lower, differences were not significant ($P > 0·05$).

### Discussion

A number of authors have raised the possibility that GH exerts direct effects on the mammary glands of ruminants (Bines *et al.* 1980; Peel *et al.* 1980; Eppard and Bauman 1984). Mepham *et al.* (1984) discussed the suggestion that GH, either directly or indirectly, affects metabolic activity of mammary secretory cells and as a result the local production of vasodilators such as kallidin and bradykinin (Dhondt *et al.* 1973). Such an effect might explain the increase in mammary blood flow during treatment of goats (Mepham *et al.* 1984) and cows (McNamara *et al.* 1983) with GH.

Although, as outlined above, increased mammary blood flow has been reported, it is considered unlikely that this is due to an effect (direct or indirect) of GH on mammary tissue. Mepham *et al.* (1984), in discussing the cause of increased mammary blood flow in goats given galactopoietic doses of GH, considered that increased blood pressure, arising from increased cardiac output, would explain the increase measured in their experiments. Recent evidence from studies with lactating cows suggests that cardiac output increases during treatment with GH (McDowell *et al.* 1984). In these studies with cows blood flow to the mammary gland and to muscle tissue of the hind limb increased by *c.* 20% — a similar increase was observed in milk production.

The present results show that local infusion of a galactopoietic preparation of GH in low doses failed to affect milk production and composition in goats and sheep. Doses of GH infused were chosen with the view to increasing local, but not peripheral, concentrations of hormone. Although no measurements were made of local tissue concentrations or concentrations in venous blood draining infused glands, it is reasonable to assert that local concentrations would have increased during infusions.

Given that the blood flow : milk yield ratio for the mammary gland approximates 500 : 1 (see Linzell 1974) and concentrations of GH in peripheral blood of 2·5–5·0 $\mu$g/l for goats and 2·5 $\mu$g/l for sheep (see Fig. 2), it is possible to estimate rates

of 'exposure' of mammary tissue to GH. In the goats, basal exposure rates would have ranged from *c.* 800 to 1600 ng/min (depending on peripheral concentration) and infusion of GH would have increased exposure by *c.* 5–30% (from 100 to 400 $\mu$g/day). In the sheep, basal rates of exposure were *c.* 280 ng/min and infusions of GH would have increased exposure from twofold (400 $\mu$g/day) to nearly tenfold (3200 $\mu$g/day).

It might be argued that interchange of blood between udder halves of the sheep and goats would have increased exposure of control glands to GH. Although it has been established that collateral circulation does occur (see Linzell 1974), it appears that interchange of blood is trivial in view of results obtained by McBride and Christopherson (1984). These workers measured blood flows of individual udder halves of lactating ewes by infusion of radiolabelled microspheres into the arterial blood supplying these individual udder halves. Insignificant amounts of radioactivity were detected in tissues of contralateral udder halves, thereby indicating trivial interchange of blood between halves of the udder.

With the exception of the period when 3200 $\mu$g/day were infused, peripheral plasma concentrations of GH were unaffected by infusions. Similarly, in the main, plasma concentrations of insulin, glucose, urea and NEFA were unaffected by GH infusions.

In connection with the latter, GH is known to exert diabetogenic and lipolytic effects (Hart 1983) and to promote muscle protein synthesis (Lindsay 1983). Indeed, it has been shown by Bines *et al.* (1980) that plasma concentrations of insulin, glucose and NEFA increase, whereas plasma urea decreases, in lactating cows exhibiting a galactopoietic response to GH. Failure to measure changes in concentrations of plasma insulin and metabolites during infusions of GH is, therefore, consistent with there being no increase in peripheral concentration of GH.

The exception to the above occurred during infusion of GH at 3200 $\mu$g/day into sheep. Plasma concentrations of GH were increased approximately threefold and there was a tendency for plasma concentrations of NEFA to increase (see Figs 2 and 3). Inexplicable increases in plasma concentrations of both GH and NEFA were measured during the last period of the sheep experiment when saline was infused into both mammary arteries.

There was a tendency for milk yields, of both GH-infused and saline-infused mammary glands, to increase during infusion of GH of 3·2 mg/day into the sheep. This dose approaches that of 0·1 mg/day per kilogram liveweight (5·5 mg/day) found by McDowell and Hart (unpublished data) to increase milk yields of lactating sheep by 10–15% when the hormone was administered as either a single subcutaneous injection each day or as a continuous intravenous infusion. Indeed, the subcutaneous injections of GH given to sheep (dose of 0·1 mg/day per kilogram liveweight) significantly increased milk yield.

Our results are consistent with results of *in vitro* studies. Skarda *et al.* (1982) found that GH did not affect synthetic activity of cultured mammary tissue from pregnant goats. Similarly, Gertler *et al.* (1983) reported that syntheses of casein, fat and $\alpha$-lactalbumin in cultured explants of bovine mammary tissue were not affected by ovine nor bovine GH. Moreover, it appears that receptors for bovine GH are not present in caprine mammary tissue (Mikulash *et al.* 1981).

In conclusion, the results of the present studies indicate that GH does not directly

stimulate the mammary gland to produce additional milk. Notwithstanding the foregoing results, it is possible that GH may affect turnover of the mammary epithelial cells and/or the biochemical activity of the epithelial cells, as suggested by Eppard and Bauman (1984), when the hormone is administered over prolonged periods. However, it should be remembered that the milk yield response to subcutaneous GH normally is measured within 1–2 days of treatment. It is also possible that a direct effect on the mammary gland may be mediated by secondary factors (e.g. somatomedins) which are subject to GH control.

## Acknowledgments

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