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## Secretion of insulin-like growth factor I and II from blood into milk of lactating goats

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### ABSTRACT

Milk from normally lactating goats contains on average 11 ng/ml IGF-I and 106 ng/ml IGF-II, as determined by specific radioimmunoassay. The present study was an attempt to determine whether IGFs in milk could have originated from the blood. <sup>125</sup>I labelled IGF-I or IGF-II was infused directly into one gland via a pudic arterial catheter for 60 min and their appearance in milk followed for 720 min. A total of 5.2% of [<sup>125</sup>I]IGF-I infused and 10.0% of [<sup>125</sup>I]IGF-II was recovered from milk from both glands. Radioactivity consisted of TCA precipitable material, which eluted from Sephacryl S-200 as free and protein-bound IGF, and TCA soluble material which probably represents degraded products of the peptides. The amount and time course of secretion of TCA soluble radioactivity in milk from both glands was similar, indicating degradation of infused peptide at extra-mammary sites. Maximum specific activity in milk from the infused gland occurred at 80-120 min for IGF-I and 120 min for IGF-II and was 2.5 fold higher than milk from the non-infused gland. The lower milk/plasma ratio for immunoreactive IGF-I compared with <sup>125</sup>I labelled IGF-I suggests all milk IGF-I might be derived from the circulation whereas similar calculations with IGF-II suggests only 78% of milk IGF-II could be accounted for by its transfer from blood.

**Keywords** Goats, IGF-I, IGF-II, milk.

### INTRODUCTION

Milk from a number of species contains appreciable quantities of insulin-like growth factor (IGF) I and II which is indistinguishable from that in plasma. In ruminants, daily administration of growth hormone increases milk concentrations of IGF-I as well as milk yield (Prosser *et al.*, 1989; Prosser *et al.*, 1991). During this time the calculated amount of IGF-I perfusing one udder half of cows increased 5-fold, thus raising the question of whether this could account for the augmented secretion of IGF-I in milk.

The purpose of the present study was to examine the kinetics of transfer of tracer amounts of [<sup>125</sup>I] labelled IGF-I and IGF-II from blood into milk of goats in an attempt to discern the origin and mechanism of secretion of these peptides into milk.

### MATERIALS AND METHODS

Six British Saanen goats, in which the external pudic artery of one gland was catheterised and the carotid artery exteriorized in a skin covered loop (Fleet and

Mephram, 1983) were used. The goats were in late lactation and anoestrus. On the day of experiment, goats were handmilked after i.v. injection of 200 mU oxytocin at 08:00, 09:00 and 10:00 h. Either [<sup>125</sup>I]IGF-I (1.3 x 10<sup>6</sup> CPM/ml) or [<sup>125</sup>I]IGF-II (1.2 x 10<sup>6</sup> CPM/ml) in sterile saline was infused via the pudic arterial catheter for 60 min beginning at 10:00 h. Milking was continued at 20 min intervals for 180 min then every 60 min until 720 min. Arterial blood was taken, via an indwelling polyethylene catheter inserted into the exteriorized carotid artery, at each milking. Radioactivity in aliquots of plasma (0.3 ml) and defatted milk (0.5 ml) were measured in a  $\gamma$ -counter (Cobra 5000, Canberra-Packard). Precipitation of undegraded [<sup>125</sup>I]IGF-I or [<sup>125</sup>I]IGF-II was achieved by addition of 10% (v/v) trichloroacetic acid (TCA) and centrifugation. The concentration of immunoreactive IGF-I in milk and plasma was determined by radioimmunoassay after acid ethanol extraction (Prosser *et al.*, 1991). Immunoreactive IGF-II was also measured by radioimmunoassay after acid ethanol extraction utilizing the procedure described by Malven, Head, Collier and

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Buonomo (1987).

Samples (2 ml) of defatted milk were applied to a 0.5 x 30 cm column of Sephacryl S-200 previously equilibrated with phosphate buffered saline, pH 7.5, containing 0.1 mg/ml BSA. Fractions of 1 ml were collected and radioactivity counted. The column was calibrated with blue dextran (Mr >200,000),  $\gamma$ -globulin (Mr 160,000), ovalbumin (Mr 45,000), [ $^{125}$ I]IGF-I (Mr 7500) and potassium dichromate (Mr 294).

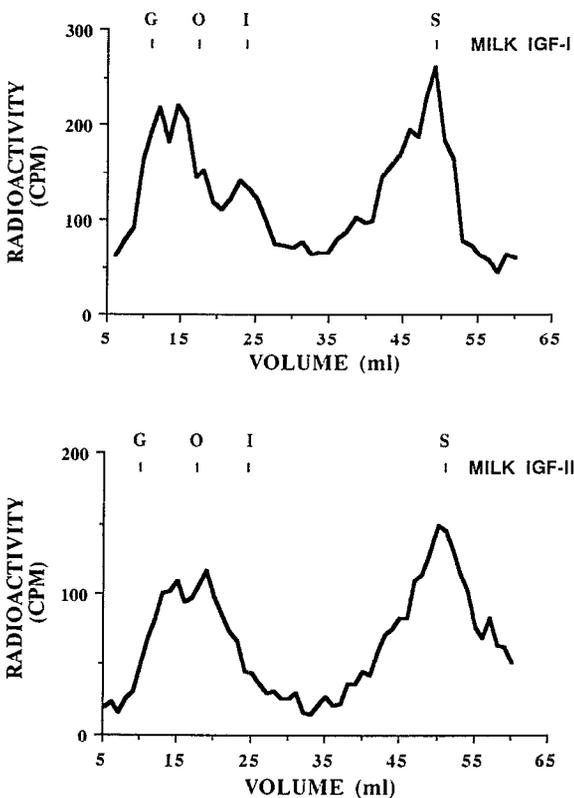
To determine whether radioactivity eluting >7500 was authentic IGF associated with binding proteins, samples of defatted milk were diluted with 1 volume of 2M acetic acid (pH 2.8), mixed and left at room temperature for 0.5 h at 4°C. They were then applied to a Sephadex G-75 column (0.5 x 30 cm) and eluted with 1M acetic acid (pH 2.5) at 0.5 ml/min. The elution position of authentic IGF-I or IGF-II was determined by chromatography of the  $^{125}$ I labelled peptide under similar conditions.

## RESULTS AND DISCUSSION

A large proportion of infused radioactivity was not taken up by the infused gland but entered into the general circulation and secreted into milk of both glands. A total of  $5.2 \pm 0.4\%$  of the [ $^{125}$ I]IGF-I infusate and  $10 \pm 4\%$  of [ $^{125}$ I]IGF-II was secreted into milk of both glands. Assuming uptake of  $^{125}$ I labelled material which escaped into the general circulation was the same for both glands, the difference between the quantity secreted into milk from the non-infused compared with the infused glands represents that which was extracted and secreted during the first passage through the infused gland. Thus, between 0-360 min  $0.4 \pm 0.09\%$  of the IGF-I and  $0.4 \pm 0.2\%$  of IGF-II infusate was secreted into milk from the infused gland on the first pass through that gland. There was no difference between glands during 360 and 720 min.

Radioactivity in milk was associated wholly with the fat-free portion of milk and consisted of TCA precipitable and soluble material. At least 82% of radioactivity derived from  $^{125}$ I labelled IGF-I and 100% of IGF-II secreted into milk during the first pass through the infused gland was TCA precipitable. The radioactivity eluted from a Sephacryl S-200 column at positions corresponding to ovalbumin, IGF and

potassium dichromate (Fig. 1). This suggests the presence in milk of IGF associated with high molecular weight in binding proteins and degraded fragments of [ $^{125}$ I]IGF or free  $^{125}$ I. When milk was acidified and then subjected to gel filtration on Sephadex G-75 at acid pH, thereby disrupting IGF/binding protein interaction, all high molecular weight material eluted as free IGF. The calculated amount of radioactivity eluting at this position was indistinguishable from the quantity of TCA precipitable material in the original sample, indicated TCA precipitation was identifying authentic IGF.



**FIG 1** Chromatograph of  $^{125}$ I labelled material in milk following intra-arterial infusion of [ $^{125}$ I]IGF-I or [ $^{125}$ I]IGF-II. Samples were eluted from Sephacryl S-200 at neutral pH. Radioactivity eluting at or near the salt peak (S) represents degraded products of IGF and were equivalent to TCA soluble radioactivity in the original sample. Radioactivity eluting near the positions of  $\gamma$ -globulin (G) and ovalbumin (O) represent IGF associated with high molecular weight binding proteins.

While all [<sup>125</sup>I]IGF-II in milk was associated with binding proteins, up to 18% of [<sup>125</sup>I]IGF-I was present as free peptide. This latter may represent the truncated form of IGF-I, isolated from bovine colostrum, which has reduced ability to associate to binding proteins (Francis *et al.*, 1988).

The presence of TCA soluble material in milk which eluted at or near the salt peak of the column, presumably reflects secretion of degraded products of IGF. Since the amount and time course of secretion of TCA soluble radioactivity into milk of both glands was similar, degradation of radiolabelled IGF most probably occurred at non-mammary sites with the products presented equally to both glands.

Changes in specific activity of <sup>125</sup>I labelled IGF-I or IGF-II in milk and plasma were calculated from the concentration of TCA precipitable material and immunoreactive IGF-I or IGF-II. Specific activity in arterial plasma increased linearly, reaching maximum at 60 min when the infusion was stopped. For IGF-I, maximum specific activity in milk from the infused gland was obtained 80-120 min after the beginning of the 60 min infusion, declining slowly thereafter (Fig. 2). The maximum specific activity for [<sup>125</sup>I]IGF-II was reached 120 min after infusion was begun and, as with IGF-I, declined slowly thereafter. In contrast, specific activity in milk from the non-infused gland reached a plateau at 120 min remaining at this level for the next 5 hours. The peak specific activity in milk from the infused gland was 2.5 fold greater than milk from the non-infused gland for both peptides.

In young goats it takes approximately 20 min for [<sup>125</sup>I]IGF-I and 60 min for [<sup>125</sup>I]IGF-II to reach maximum in lymph draining the foreleg following their injection i.v. (Prosser, Baucells and Fleet, unpublished observations). Much, if not all, of this delay may be accounted by the time required for internalization and release of IGF by endothelial cells (Bar *et al.*, 1986). Assuming a similar mechanism occurred during transfer of IGF-I and IGF-II from blood into interstitial fluid in the mammary gland, it would appear a major barrier for secretion of IGFs into milk was the vascular endothelium.

The time course of transfer in milk, however, indicates a more complex mechanism. Specific activity in milk from the infused gland declined slowly after attaining its maximum, to reach values similar to that in

milk from the non-infused gland some 5-6 hours after infusion had stopped. Since each gland received the same supply of circulating IGF once infusion was stopped this difference between glands must represent IGF already taken up by the infused gland but which only appeared in milk after some delay.

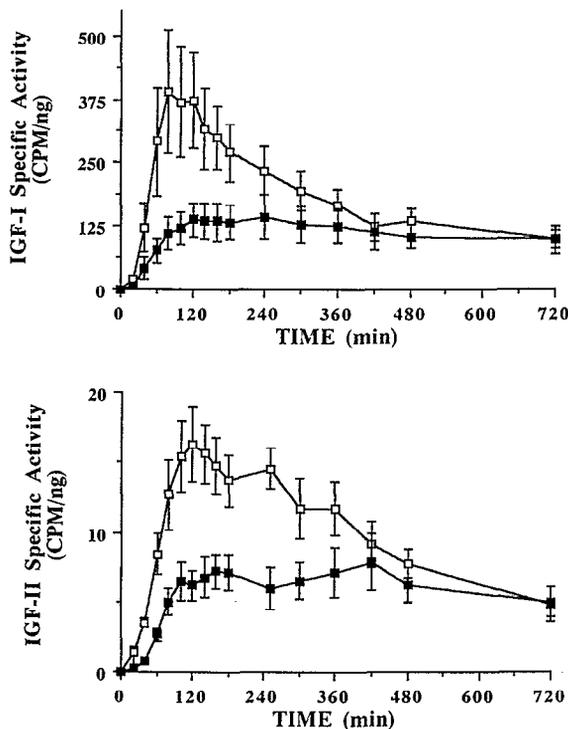


FIG 2 Time course of the change in specific activity of <sup>125</sup>I labelled IGF-I and IGF-II in milk from the infused (□) and non-infused (■) gland. Specific activity was calculated from the amount of radioactivity precipitated by 10% TCA and immunoreactive IGF present in the samples.

Secretion of blood-borne constituents into milk may be achieved by a non-selective passage across the mammary epithelium between cells (paracellular pathway) or a more selective uptake and transfer through cells (transcellular pathway; Linzell and Peaker, 1971). Transfer of substances into milk via the former pathway occurs rapidly compared with the latter which requires time for uptake and secretion. Thus, while it is not possible to unequivocally state that the mechanism of

transfer of circulating IGF-I and IGF-II into milk does not involve the paracellular pathway we suggest the major entry is via the transcellular route.

To determine whether immunoreactive IGF present in milk originated from the circulation the milk to plasma ratio of immunoreactive IGF was compared to labelled IGF. Data for these calculations was derived from transfer into milk from the non-infused gland over the period from 180 to 720 min, since after 180 min labelled IGF in the general circulation had attained a similar distribution pattern with binding proteins as endogenous immunoreactive IGF and was assumed to behave similarly. The milk:plasma ratio for immunoreactive IGF-I was 0.04 and 0.12 for  $^{125}\text{I}$  labelled IGF-I. The ratio for immunoreactive IGF-II was 0.26 and 0.20 for  $^{125}\text{I}$  labelled IGF-II. Thus it appears that all of the IGF-I but only 78% of IGF-II in milk may be accounted for by its transfer from the circulation.

When unlabelled IGF-I was included in the [ $^{125}\text{I}$ ]IGF-I infusate the specific activity in milk from the infused gland was reduced to that of the non-infused gland, indicating a competitive and saturable mechanism of secretion and reinforcing the concept for selective uptake and secretion of this peptide. In contrast, transfer of [ $^{125}\text{I}$ ]IGF-II was not affected by inclusion of unlabelled IGF-II or IGF-I in the infusate.

The significance of the present findings demonstrating transfer of blood-borne IGF-I and IGF-II into milk is two fold. Firstly it shows that all IGF-I and most IGF-II in milk could be derived from the circulation. A number of hormones and growth factors present in the circulation are present in milk and amongst these progesterone (Heap *et al.*, 1975), oestrone sulphate (Heap *et al.*, 1984), prolactin, EGF (Brown *et al.*, 1986) and now IGF-I and IGF-II have been shown to be transferred from the circulation. Secondly, these experiments demonstrate the potential of the mammary gland in assessing effects of modifications to either IGFs or binding proteins on transfer into tissues *in vivo* and mechanisms involved in their transfer. It is notable that IGF-I is also able to elicit a biological response in the mammary gland, in terms of increased milk secretion and mammary blood flow (Prosser *et al.*, 1990).

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## REFERENCES

- Bar R.S.; Boes M.; Yorek M. 1986. Processing of insulin-like growth factors I and II by capillary and large vessel endothelial cells. *Endocrinology* **118**: 1072-1080.
- Brown K.D.; Blakeley D.M.; Fleet I.R.; Hamon M.; Heap R.B. 1986. Kinetics of transfer of  $^{125}\text{I}$ -labelled epidermal growth factor from blood into mammary secretion of goats. *Journal of Endocrinology* **109**: 325-332.
- Fleet I.R.; Mephram T.B. 1983. Physiological methods used in the study of mammary substrate utilization in ruminants. In *Biochemistry of Lactation*. Ed. Mephram T.B. Amsterdam-New York: Elsevier. pp 469-492.
- Francis G.L.; Upton F.M.; Ballard F.J.; McNeil; Wallace J.C. 1988. Insulin-like growth factors 1 and 2 in bovine colostrum: sequences and biological activities compared to a potent truncated form. *Biochemical Journal* **251**: 95-103.
- Heap R.B.; Bedford C.A.; Linzell J.L. 1975. Metabolic clearance rate, production rate and mammary uptake of progesterone in the goat. *Journal of Endocrinology* **64**: 485-502.
- Heap R.B.; Hamon M.; Fleet I.R. 1984. Transport of oestrone sulphate by the mammary gland in the goat. *Journal of Endocrinology* **101**: 221-230.
- Linzell J.L.; Peaker M. 1971. Mechanism of milk secretion. *Physiological Reviews* **51**: 564-597.
- Malven P.V.; Head H.H.; Collier R.J.; Buonomo F.C. 1986. Periparturient changes in secretion and mammary uptake of insulin and concentrations of insulin and insulin-like growth factors in milk of dairy cows. *Journal of Dairy Science* **70**: 2254-2265.
- Prosser C.G.; Fleet I.R.; Corps A.N. 1989. Increased secretion of insulin-like growth factor I into milk of cows treated with recombinantly derived bovine growth hormone. *Journal of Dairy Science* **56**: 17-26.
- Prosser C.G.; Royle C.; Fleet I.R.; Mephram T.B. 1991. The galactopoietic effect of bovine growth hormone in goats is associated with increased concentrations of insulin-like growth factor I in milk and mammary tissue. *Journal of Endocrinology* (in press).
- Prosser C.G.; Fleet I.R.; Corps A.N.; Froesch E.R.; Heap R.B. 1990. Increase in milk secretion and mammary blood flow by intra-arterial infusion of insulin-like growth factor I into the mammary gland of the goat. *Journal of Endocrinology* **126**: 437-443.