
THE PROCEEDINGS OF THE
NEW ZEALAND SOCIETY
OF
ANIMAL PRODUCTION
VOLUME 62 2002

The time-course of milk yield and hormonal responses following growth hormone injections in hourly-milked goats

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ABSTRACT

Five goats were milked hourly for 43 h on two occasions, on one occasion receiving injections of growth hormone (GH; 15 mg S.C.) after 4 and 28 h of the milking period and on the other occasion were untreated. Blood samples were taken before each milking, the latter facilitated by intravenous injection of oxytocin (100 m i.u.).

Milk yield began to rise 4-5 h after the injection of GH and continued to rise for a further 8-9 h until a level of production 18% above the pre-treatment yield was achieved. Following the second injection of GH the yield increment was reduced such that by the end of the experiment the yield increase was 24%. In one goat the milk yield response was not sustained and these data were excluded from the above calculations.

Plasma insulin-like growth factor-I (IGF-I) and insulin concentrations increased in a similar time-course to the change in milk yield following GH treatment but were relatively constant in control experiments. IGF-I and insulin content of plasma was approximately doubled by GH treatment by the end of these experiments.

Keywords Growth hormone, milk yield, hormones, lactation, goats, insulin-like growth factors.

INTRODUCTION

The physiological changes induced by growth hormone (GH) which elicit the increase in milk yield following injection of GH into ruminants have not been defined. However, it is likely that the effect of GH is not directly on mammary tissue but is mediated by a number of secondary endocrine and metabolic changes among which are increased plasma and mammary concentrations of insulin-like growth factor-I (Davis *et al.*, 1988; Prosser *et al.*, 1990).

The objective of the experiment described below was to establish the time-course of the milk yield response to GH treatment in goats. While the effect of GH on milk yield is known to occur usually within 24 hours of GH injection (Mepham *et al.*, 1984) a more exact description of the temporal changes in plasma hormones and milk yield is important information for the purpose of studying mediation of the GH effect on milk yield. Among the putative mediators of GH action there is evidence that insulin-like growth factor-I (IGF-I) is involved in assisting the milk yield response (Prosser *et al.*, 1990) and hence was of primary interest in this study.

MATERIALS AND METHODS

Animals

Five multiparous Saanen goats in mid-lactation were used. They were housed individually in pens and fed a diet of 2.5 kg concentrates daily with chopped hay offered *ad libitum*. Routine milking was carried out twice daily at 07.30 h and 15.30 h.

Experimental Protocol

On the day before each experiment all goats were milked 5 times (at 3-5 h intervals) in conjunction with intravenous administration of oxytocin (2 x 100 m IU Oxytocin-S, Intervet Pty Ltd, Lane Cove, NSW, Australia). The last milking was carried out at 23.00 h.

On the day of the experiment all goats were milked out at 04.00 h and at hourly intervals for the next 43 h (all milkings with oxytocin - as above). Two goats received subcutaneous injections of GH (15 mg) at 4

TABLE 1 Milk yield of goats during the experiments where growth hormone was given by subcutaneous injection (15 mg) at 4 h and 28 h. Figure 1 shows the time-course of the milk yield increase.

Goat No.	Pre-experiment milk yield (ml/h)[1]	Milk yield (ml/h) 1-5 h	16-20 h	36-40 h	Maximum response (%)
5	99	106 (5)[2]	127 (2)	130 (5)	23
6	84	102 (5)	109 (4)	104 (8)	2
8	66	73 (4)	86 (3)	93 (4)	27
9	64	64 (5)	77 (4)	81 (2)	27
10	136	137 (4)	156 (4)	162 (5)	18
Mean[3]	91 (17)	95 (17)	112 (18)	117 (18)	24 (2)

[1] Mean secretion rate over 3 d of twice-daily milking

[2] Standard error of the mean in parenthesis

[3] Mean excluding data from goat 6

and 28 h and the remaining 3 goats were not treated. This protocol was repeated 2 weeks later with the previously untreated goats receiving GH as above, the remaining goats being untreated.

Sampling

Blood samples (10 ml) were taken hourly throughout the experiment into tubes containing ethylenediamine tetraacetic acid (EDTA) as anticoagulant and plasma harvested and stored at -20°C. Milk samples were taken hourly for fat, protein and lactose assay by infra-red reflectance spectroscopy (Foss Milkoscan 104; Foss Electric, Hillerod, Denmark).

Hormone Assays

IGF-I in blood plasma was determined by radioimmunoassay as described by Spencer, Hodgkinson and Bass (1991) using the acid/ethanol extraction procedure described by Breier *et al.* (1991). Serial dilution of goat plasma showed good parallelism with the standard curve and recoveries of added IGF-I were 91±4.2 (sem)%. Assay sensitivity was 0.15 ng/ml and intra- and inter-assay coefficients of variation were 3.9% and 9.1% respectively. Cross reactivity of the antiserum with IGF-2 was less than 1%.

Plasma insulin content was determined using an insulin assay kit for human plasma (Coat-a-count, Diagnostic Products Corporation, Los Angeles, California 90045, USA). This assay was validated for use with goat plasma, serial dilutions giving a parallel response to the standard curve and recovery of added insulin varying from 95 to 100%. Inter- and intra-assay coefficients of variation were 7.5 and 5% respectively. Because the use of EDTA as anticoagulant interferes with this assay (giving an enhancement of plasma insulin concentration) plasma values were corrected for the effect of EDTA.

Statistical Methods

In order to facilitate assessment of the time-course of the milk yield responses the data were smoothed using a resistant smoother function (Minitab Inc., State College, Pennsylvania 16801, USA).

RESULTS

Milk Yield

In GH-treated goats milk yield began to increase at 8-9 h following GH injection at 4 h (Fig. 1). This increase continued for a further 8-9 h until a level of production

20-25% above the pre-experiment milk yield was achieved. This increase was sustained until a further increase in milk yield occurred beginning at 36-37 h following the second injection of GH at 28 h.

with the yield continuing to increase to the end of the experiment (Table 1).

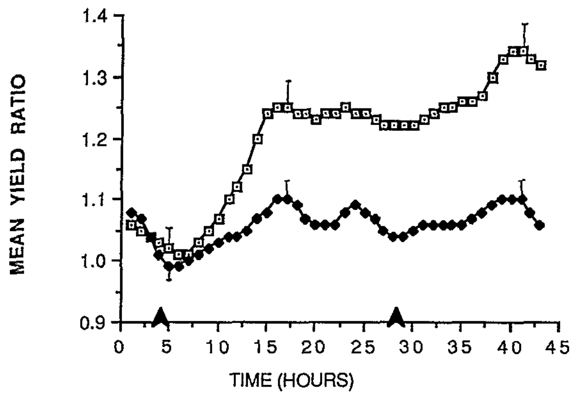

FIG 1 Hourly milk yield of growth hormone (GH)-treated (▫—▫) and control (◆—◆) goats. Milk yields are expressed as a ratio of the previous milk yield (mean of 3 d before the start of hourly milking). Data shown are the smoothed averages of four goats excluding data from goat 6 (Table 2) where the milk yield increase to GH treatment was not sustained. Growth hormone treatments (15 mg S.C.) were given at the times indicated by the arrows.

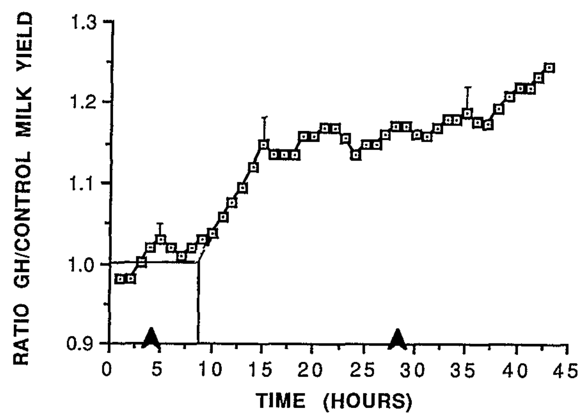

FIG 2 The time course of the milk yield response of goats to growth hormone (GH) treatment. Milk yield is expressed as the ratio of the mean milk yield during GH treatment to that obtained in control experiments (as shown in Figure 1). Interpolation on the graph as shown indicates that the rise in milk yield begins at 8-9 h and at 37 h, 4-5 and 9 h after the first and second injections of GH (arrowed).

Milk yield of both treated and control goats fell 5-10% over the first 6 h of the experiment, but thereafter in control goats mean yield fluctuated at a level 5-10% greater than that observed in the pre-experimental period (Fig.1).

Although there was a small increase in milk yield in goat 6 following the first GH injection this increase was not sustained (Table1). Data from this goat were not used in the preparation of Figs 1 and 2.

The milk yield response (expressed as a ratio of GH-treated/control experiments) is shown in Fig. 2. Interpolation on this graph indicates that the milk yield response to injection of GH began at about 9 h, 5 h after the first injection of GH. However, after the second injection of GH, the milk yield increase began 9 h later.

The percentage increase in milk yield attributable to GH averaged 18% by 16-20h and 22% by 36-40 h

Plasma IGF-I

The mean response of plasma IGF-I concentration to GH injection is shown in Fig. 3 for the goats showing a milk yield response. Plasma IGF-I content began to increase after 8-9 h, 4-5 h after the injection of GH. The initial rise in plasma IGF-I content was from a mean (Table 2) of 161 ng/ml to 243 ng/ml at 16-20 h and 277ng/ml at 36-20 h. There was a close temporal relationship between the rise in milk yield (Fig. 1) and change in plasma IGF-I content (Fig. 3).

Variation in the increment in plasma IGF-I concentration was not associated with variation in the milk yield response to GH injection in either absolute or percentage terms (Table 1). In goat 6 where there was no milk yield response to GH injection there was still a marked rise in plasma IGF-I content. In control goats plasma IGF-I content was remarkably constant throughout the experiment at 150-160ng/ml.

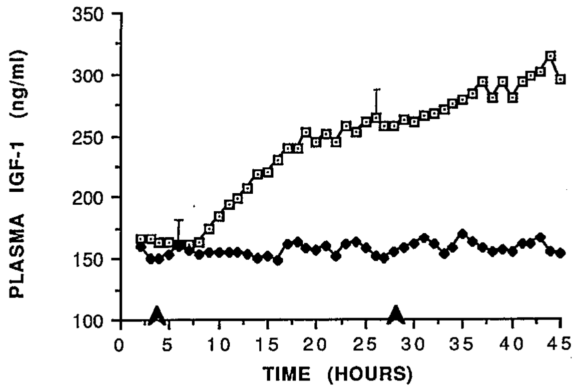

FIG 3 Change in plasma insulin-like growth factor (IGF)-I concentrations in goats (n=5) treated with growth hormone (□—□; 15 mg S.C., arrowed) or untreated (◆—◆). Blood sampling began at the time of the second milk yield determination. Variation in IGF-I responses between goats are shown in Table 2.

TABLE 2 Plasma insulin-like growth factor-I (IGF-I) content during 3 periods of sampling. Growth hormone (15 mg) was given subcutaneously at 4 h and 28 h. Plasma IGF-I content began to increase after 8-9 h (refer Fig. 2).

Goat No.	Plasma 1-5 h	IGF-I concentration (ng/ml) 16-20 h	36-40 h	Maximum response (%)
5	203 (2)[1]	263 (10)	289 (5)	43
6	147 (3)	228 (5)	241 (4)	64
8	149 (1)	246 (6)	307 (6)	106
9	114 (2)	189 (4)	195 (6)	71
10	193 (4)	287 (7)	354 (3)	83
Mean	161 (16)	243 (16)	277 (27)	73 (10)

[1] Standard error of the mean in parenthesis

Plasma Insulin

Plasma insulin concentrations began to rise at about the same time as milk yield and plasma IGF-I content and peak concentrations (almost double pre-GH injection) were achieved by 20 h (Fig. 4). There was a further increase in plasma insulin content towards the end of the experiment by which time the insulin content of plasma of GH-treated goats was almost 3 fold that of control goats.

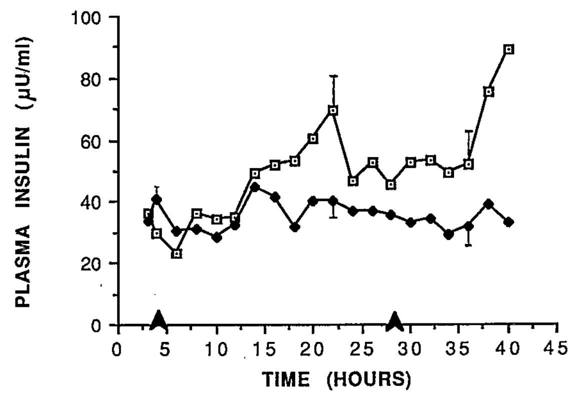

FIG 4 Change in plasma insulin concentrations with time during experiments where goats (n=5) were treated with growth hormone (□—□;15 mg S.C., arrowed) or were untreated (◆—◆).

In one goat (goat 5) plasma insulin content was unchanged until after 30 h (relative to control; data not shown) while milk yield increased by over 20%. In goat 6 which did not show a sustained milk yield response plasma insulin content was increased.

In control goats plasma insulin content was relatively stable throughout the experiment at 30-35 mU/ml.

DISCUSSION

After GH injection of lactating ruminants it is likely that several endocrine and metabolic changes occur which act in concert to enhance milk production. These changes include increased plasma IGF-I content (Davis *et al.*, 1987), increased and decreased IGF binding protein content in plasma (Cohick *et al.*, 1990; Vicini *et al.*, 1990), increased mammary blood flow (see Davis *et al.*, 1988), increased mammary thyroxine 5' mono-deiodinase activity (Capuco *et al.*, 1989), increased plasma insulin content (Davis *et al.*, 1987) and increased glucose and free fatty acid turnover in plasma (Bauman *et al.*, 1988).

The present study defined the time-course of some of these changes and showed a high degree of synchrony between changes in plasma IGF-I and insulin concentration and change in milk yield following GH injection. These data confirm the candidacy of IGF-I in the mediation of GH action on milk production. However the goat which failed to sustain a milk yield response to GH still gave a 'normal' response in plasma IGF-I and insulin concentration, illustrating that change in plasma IGF-I does not necessarily result in increased milk output. Further, in goat 5 where milk yield and plasma IGF-I content were both increased by GH treatment there was no change in plasma insulin concentration over the time when milk yield increased by 20%. Such data do not support a controlling influence of plasma insulin in the mediation of GH action on milk production.

The use of hourly-milked goats for studies of this type have been hampered by the milk yield response to increased milking frequency (Wilde *et al.* 1991). This effect was eliminated by frequent milking on the day before the experiment which resulted in milk yield of control goats being relatively stable throughout the 43 h of the experiment. Coefficients of variation in milk yield over the course of the control experiments varied between goats from 4% to 10%. The assessment of changes in milk secretion rate were aided by application of statistical smoothing procedure (a combination of running medians and running averages) which is an appropriate technique where there is likely to be correlation between adjacent data points, arising in this case because of variation in the efficiency of milk removal at each milking.

The milk yield response to GH injection was not achieved in its entirety by a single injection of GH (Fig. 2). A number of studies have reported that milk yield (of cows) continues to increase for several days following the commencement of daily GH injections (eg. Machlin 1973). In Fig. 1, the data show that following an initial rapid milk yield response to GH injection, the response to the second injection is attenuated. The slow increase in yield response to a maximum observed in other studies is probably indicative of mammary secretory cell hypertrophy to accommodate the increased stimulus to produce milk. The acute rise in production shown in Fig. 1 probably represents a metabolic response in the secretory cell perhaps associated with enhanced glucose transport into the mammary cell as suggested by the data of Mepham *et al.* (1990).

ACKNOWLEDGEMENTS

The excellent technical assistance of Roger Dean, David Phipps and Bill Edgely is gratefully acknowledged. Growth hormone used in these experiments was recombiant bovine somatotropin, a gift from American Cyanamid Company, New Jersey, U.S.A.

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