EFFECTS OF THYROXINE AND GROWTH HORMONE TREATMENT OF DAIRY COWS ON MILK YIELD, CARDIAC OUTPUT AND MAMMARY BLOOD FLOW

S. R. Davis, R. J. Collier, J. P. McNamara, H. H. Head and W. Sussman

University of Florida, Gainesville 32611

ABSTRACT

Four cows received thyroxine injections (T4; 20 mg/d) and three cows received growth hormone injections (GH; 44 mg/d) for 4 d during successive 16-d experimental periods. Measurement was made of milk yield, protein yield, mammary tyrosine and phenylalanine uptake, blood plasma hormone concentrations, mammary blood flow and cardiac output. Milk yield increased by 25% with T4 and 21% with GH treatment. Milk protein content tended to decline during T4 treatment and increase following GH treatment. Cardiac output increased by 8.9 liter/min (20%) and 4.6 liter/min (10%) with T4 and GH injection. Mammary blood flow (half-udder) increased from 3.6 to 4.9 liter/min (35%) and from 3.3 to 4.4 liter/min (33%) with T4 and GH treatment, respectively. These increases calculated on a whole-udder basis, accounted for 28% (T4) and 48% (GH) of the increases in cardiac output. The proportion of cardiac output perfusing the (whole) udder increased to 19.1% (T4) and 18.7% (GH), increases of 17 and 30%, respectively. Heart rate increased with T4 (but not GH treatment) from 80 to 115/min. Ratio of blood flow to milk yield was not changed by either treatment. The proportion of cardiac output perfusing the udder likely plays a major role in facilitating the partitioning of nutrients for milk synthesis.

(Key Words: Somatotropin, Thyroxine, Cows, Milk Yield, Mammary Glands, Blood Flow.)

Introduction

Rate of milk production in cows may be increased by administration of growth hormone (GH) or thyroxine (T4), either by sc injection or via feed as iodinated protein (Meites, 1961; Hart, 1983; Collier et al., 1984; Davis and Bass, 1984; Bauman and McCutcheon, 1985). However, little is known of the mechanisms by which these hormones achieve their effects.

The effect of GH on milk production of cows is associated with preferential partitioning of nutrients for milk synthesis (Tyrrell et al., 1982) and little change in feed intake during short-term treatment (Bauman and McCutcheon, 1985). In contrast, response to T4 treatment is associated with increased feed intake and no change in the efficiency of conversion of feed to milk (Thomas, 1953).

The following experiments were carried out to investigate the cardiovascular effects of GH and T4 on lactating dairy cows and the possible role of mammary blood flow in facilitating the partition of nutrients for milk synthesis. A companion paper describes the effects of GH and T4 on mammary uptake of glucose, oxygen and substrates for milk fat synthesis (Davis et al., 1987).

Materials and Methods

Animals, Housing and Diet. Mature Jersey cows (table 1) were housed in pairs in controlled-environment chambers at a constant 20 C, 80% humidity and under a 16 h light: 8 h dark lighting regimen.

Daily feed allocation was sufficient to permit ad libitum intakes (>10% residue) and was offered once daily. Diet was based on cottonseed hulls, corn and soya bean meal (table 1). Calculated net energy (NEE) was 6.25 MJ/kg, acid

1Florida Agric. Exp. Sta. Publ. No. 6522. The support of the New Zealand-United States Technical Cooperation Program and the Upjohn Co., Kalamazoo, MI in funding this work is acknowledged gratefully. We also thank R. F. Olsen of the Upjohn Co. for the somatotropin assays.
3To whom reprint requests should be directed. Present address: Monsanto Co., 700 Chesterfield Parkway, BB3F, St. Louis, MO 63189.
5Dairy Sci. Dept.
Received March 10, 1986. Accepted July 17, 1987.
TABLE 1. EXPERIMENTAL ANIMALS

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</table>

*Diet composition (kg/t): cotton seed hulls, 342 kg; crimped corn, 427 kg; soybean meal, 195 kg; ground lime-
stone, 9.1 kg; vitamins A, D and E, 9.1 kg; calcium phosph-
ate 8.7 kg; KCl, 4.2 kg; trace mineral salt, 2.7 kg; MgO,
2.2 kg.

A polyvinyl catheter (.86 mm id × 1.52 mm od) was placed in the external maxillary artery and exteriorized on the neck. A further polyvinyl catheter (1.3 mm id × 2.3 mm od) was placed in the left sc abdominal vein and exteriorized on the left flank. Both catheters were filled with heparinized saline and maintained in polyethylene packs as described.

Cardiac Output Determination. Cardiac output was determined using a Swann-Ganz ther-
modilution catheter7 (7F) inserted into the pul-
monary artery via a jugular vein. Positioning in
the pulmonary artery was determined from
blood pressure measured at the catheter tip.
A separate catheter (8F; 108 cm) for saline
injection was inserted through a 10-gauge nee-
dle into the ipsilateral or contralateral jugular
vein; its tip was positioned just outside the right
atrium. This catheter permitted the injection of
30 ml cold (0 to 5 C) saline over 3 to 4 s. both
catheters were filled with heparinized saline and
protected in polyethylene packs.
Cardiac output was calculated following
saline injection using a cardiac output com-
puter7 (model 9502) and the Steward-Hamilton
Equation (Muir et al., 1976). The estimate of
cardiac output was the average of 5 to 10 deter-
minations made over a period of 10 to 15 min.

Mammary Blood Flow Measurement. Be-
cause of ground electrical interference, the
cows were isolated from the concrete floor each
morning on dry rubber mats placed on plastic
sheets. Mammary blood flow was measured
continuously by connection of the probe to the
flowmeter9. Each flow probe was calibrated in
vitro before and after use by pumping saline
through an isolated artery at known flow rates
(Roman-Ponce et al., 1981). The calibration
factor determined was pre-set on the flowmeter
so that actual mean flow rates were recorded
continuously on the chart recorder. Heart rates
were determined remotely with the flowmeter
set in pulsatile mode.
An additional estimate of mammary blood
flow was obtained using a Fick principle
method based on comparison of milk
phenylalanine and tyrosine output with their re-
spective arterial-venous (AV) differences.
Mammary blood flow was calculated as fol-
lows:

\[
\text{blood flow} = \frac{\text{milk tyrosine output}}{\text{phenylalanine output}} \times \frac{100}{(A-V)} (100-HcT)
\]

6Biotal, Bio-Ceutic Lab., Inc., St. Joseph, MO.
7Edwards Lab., Inc., Santa Ana, CA.
8C & C Instruments, Culver City, CA.
9Narco Inc., Dallas, TX.
where flow was expressed as liter/min, A-V was mammary arterial-venous concentration difference for tyrosine or phenylalanine and HcT was hematocrit (%). Milk tyrosine and phenylalanine output were calculated assuming that 92% of milk protein was synthesized in the mammary gland and tyrosine and phenylalanine concentration in milk protein were 4.9 and 5.1 g/100 g, respectively. The criteria and assumptions for the calculations are presented by Davis and Bickerstaffe (1978).

Daily Sampling and Measurement Routine. The cows were milked and fed at 0800 and continuous blood flow monitoring began between 0900 and 0930. Simultaneous arterial and mammary venous blood samples (four pairs) were taken at 15- to 20-min intervals beginning at 1000, following inflation of the Fogarty catheter to occlude the external pudic vein. Plasma was harvested following centrifugation and stored at -20 C.

Blood and Milk Analyses. Plasma tyrosine was assayed fluorometrically according to the method of Waalkes et al. (1957). Plasma phenylalanine concentrations were determined by kit.

Milk protein content was determined using a Coomassie Blue dye-binding method calibrated against Kjeldahl nitrogen determinations. Plasma GH concentration was determined as described by Moseley et al. (1982); prolactin as described by Eley et al. (1981) and insulin, T4 and triiodothyronine (T3) as described by Collier et al. (1982).

Experimental Design, Treatments and Contingency Procedures. Following surgery a 7-d recovery period was allowed before experimental measurements began. Measurements were then made over 32 d in the sequence: 4 d control, 4 d treatment, 8 d recovery, 4 d control, 4 d treatment, 8 d recovery. For the first pair of cows the first treatment was GH (bovine) 44 mg/d for 4 d by sc injection; (dissolved according to Peel et al., 1981) followed (12 d later) by T4 treatment (20 mg/d for 4 d by sc injection; dissolved in 20 ml alkaline saline). For the second pair of cows the treatment sequence was reversed.

As far as possible the routine for daily measurements was adhered to as described. However, because of catheter and(or) equipment failures, data were not collected on 2 or 3 d of each experimental period for each cow. The sc abdominal vein catheters failed in all cows to 4 wk and were replaced with polyethylene catheters. Facial artery catheters failed in two cows and were replaced by catheterization of an intercostal artery under local anesthesia. Two Fogarty catheters burst in situ and were withdrawn but not replaced.

Animal Health. Rectal temperatures were measured at the beginning and end of each working day and any increase above normal was treated immediately with an im injection of penicillin-streptomycin. No experimental measurements were taken if morning rectal temperature was elevated and(or) feed intake was depressed. Antibiotic was administered routinely for 4 d following surgery.

Cow 3 (table 1) aborted following completion of the T4 injections but before the start of the GH injection. The abortion was accompanied by severe mastitis and this cow was withdrawn from the trial. A replacement animal suffered a displaced abomasum during GH treatment. Data are available for three cows treated with GH.

Statistical Analysis. Experiments consisted of 16-d periods. Data for the four initial control days were averaged across days and cows. Between d 5 and 16, a cubic polynomial was fitted for each animal and each variate and the average fitted curve for each variate joined to the control period mean. Thus the data presented in figures 1 to 4 are the 4-d control averages and the means of the individual fitted curves. This pooled curve was adapted to permit concise display of data and to overcome problems of missing data which arose, for example, following catheter failure. For all characteristics and cows, data were obtained for a minimum of 11 d during the 16-d experimental period. For some parameters daily data collections were complete (milk yield and composition).

Statistical comparisons were made at the point of maximum deviation of the fitted curve from the control period mean for each animal. Significance of difference was assessed by Student’s T-test. The rationale behind this approach has been discussed (Rowell and Walters, 1976).

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10 Kit 60-F. Sigma Chemical Co., St. Louis, MO.
11 Biorad Lab., Richmond, CA.
12 Med-Tech Inc., Elwood, KS.
Results

Milk Yield, Milk Protein and Dry Matter Intake. Milk yield (left-half) increased from 7.9 to 9.9 kg/d (25%) following T4 treatment (P<.05) and from 8.1 to 9.8 kg/d (21%) following GH treatment (P<.01; figure 1a). Peak responses were obtained on d 10 and 8 for T4 and GH, respectively. Milk yield response did not differ between udder halves. Milk protein percent declined during the period of T4 and GH treatment but increased following GH injections, although changes were not significant (figure 1b). Milk protein yield (half-udder) increased (figure 1c) from 301 to 358 g/d (19%) following T4 treatment (P<.10), and from 298 to 375 g/d (26%) with GH treatment (P<.05). Maximum protein yield occurred later (d 10) than maximum milk yield in GH-treated cows. In T4-treated cows, maximum protein yield was earlier than maximum milk yield as milk protein content declined following T4 injections (figure 1b).

Dry matter intake was high throughout each experiment, varying between 17.5 and 20.7 kg/d (5 to 6% of live weight).

Cardiac Output and Mammary Blood Flow. Cardiac output and mammary blood flow increased in T4- and GH-treated cows (figure 2a, b). Cardiac output increased from 45.4 to 54.3 liters/min (20%; P<.05) in T4-treated cows, reaching a maximum on d 10. Cardiac output in GH-treated cows also reached a maximum on d 10, increasing from 46.2 to 50.8 liters/min (10%; P<.01), and returned to control values by the end of the 16-d experimental period with each treatment.

Mammary blood flow (half-udder) increased from 3.6 to 4.9 liter/min (35%; P<.01) associated with injections of T4, and from 3.3 to 4.4 liter/min (33%; P<.01) associated with injections of GH, with maxima occurring on d 8 and over d 8 to 10, respectively (figure 2b). Calculated on a whole-udder basis, increases in mammary blood flow accounted for 28% (T4) and 48% (GH) of the increases in cardiac output.

During control periods the proportion of cardiac output perfusing the whole udder was 16.3% (T4) and 14.4% (GH). Following treatment these proportions increased to 19.1% (T4) and 18.7% (GH; P<.05), respectively (figure 2c). These elevated proportions declined to control levels by the end of the 16-d experimental periods.

Blood flow: milk yield ratio increased rapidly with T4 treatment, from 666 to 766 (d 6), and more gradually following GH treatment, from 587 to 669 (d 12). These increases were not statistically significant (figure 2d).

Following T4 treatment, heart rate increased from 80 beats/min during the control period to a maximum of 115 beats/min on d 9 (P<.001), thereafter declining to control values by d 16. With GH treatment there was a small, but not significant, increase from 78 to 85 beats/min on d 9.

From these data it can be calculated that stroke-volume declined from .57 to .47 liters (P<.01) in response to T4 treatment but was unchanged by GH treatment at .59 and .60 liters
Figure 2. Cardiac output (a), mammary blood flow (left-udder; b), mammary blood flow (whole udder) as a proportion of cardiac output (c) and ratio of blood flow to milk yield (d) during T4 (---) and GH (-----) experimental periods. See legend to figure 1 for statistical details.

for control and maximum response, respectively.

Mammary Tyrosine and Phenylalanine Uptake. The arterial plasma concentration of phenylalanine increased from 11.4 to 14.7 µg/ml (29%; P<.001) by d 10 in response to T4 treatment, returning to control values by d 16. Neither plasma tyrosine or phenylalanine content was affected by GH treatment (figures 3a, b). The AV difference of phenylalanine and tyrosine did not change due to treatment (figures 3c, d).

Mammary extraction (%) of phenylalanine and tyrosine was unaffected by treatment, averaging 30 ± 1 and 36 ± 2 during the T4 treatment period (n = 50) and 34 ± 1 and 40 ± 1 during the GH experiment period (n = 41). The ratio of plasma uptake to milk output for tyrosine was .92 ± .07 during both experimental periods and for phenylalanine was .88 ± .04 during the T4 experiment (n = 50) and .75 ± .07 during the GH experiment (n = 41). Ratios were unaffected by treatment.

Calculated Mammary Blood Flow from Mammary Tyrosine and Phenylalanine Uptake. Mammary AV difference of tyrosine and phenylalanine was used to calculate mammary blood flow by a Fick principle method. There were no significant differences between flow estimates derived from mammary tyrosine or phenylalanine AV difference, thus these data were pooled. The Fick blood flow estimate equated closely with EM estimates (table 2), verifying the in vitro calibration of the flow cuffs. Pooled correlation of Fick flow with EM flow was .54 (P<.001, n = 89).

Blood Plasma Hormone Concentrations. Injection of GH increased plasma GH concentration threefold in samples taken 20 to 22 h after injection, while T4 injection was associated with a small but nonsignificant rise in plasma GH (figure 4a). Plasma T4 concentration increased 15-fold and T3 sixfold (P<.001) in response to T4 injection; maxima occurred by d 7. Concentrations of plasma T3 and T4 (figures 4b, c) were not affected by injections of GH.

While GH injections were associated with an increase in plasma insulin content, the response was not statistically significant, occurring in only two of three treated cows. In contrast, T4 injections reduced (P<.01) plasma insulin concentration, the minimum occurring on d 8, a decrease of 55% from control concentrations (figure 4d).

Blood plasma prolactin concentrations were unaffected by either treatment being 17 ± 4
ng/ml during the T4 experiment (average across cows) and 24 ± ng/ml during the GH experiment.

Discussion

Jersey cows used in this study were high producers relative to their body weight (350 kg). The milk production responses elicited by four daily injections of GH (44 mg/d) or T4 (20 mg/d) were similar (20 to 25%) and were sustained for several days following cessation of injections.

Increases in milk yield were similar to those obtained previously (Meites, 1961; Collier et al., 1984). It is unlikely that the responses to treatment were maximal. Machlin (1973) reported that increases in production continued to accumulate for up to 10 d of GH treatment; Eppard et al. (1985) indicated that the maximum response in Holstein cows was obtained with a daily GH dose of almost 80 mg.

The decline in milk protein content observed in response to T4 injection, although not statistically significant, has been observed previously (Blaxter et al., 1949). A decline in milk protein content observed during GH injection was dose-responsive (Eppard et al., 1985). However, during the post-injection period in our study milk protein content tended to increase.

Mammary Tyrosine and Phenylalanine Uptake. Tyrosine and phenylalanine have been classified as essential amino acids, indicating that mammary uptake from blood equals output in milk protein (Mepham, 1982). This classification was supported by this study and, further, the ratio was unaffected by treatment.

In spite of a substantial increase (30 to 35%) in mammary blood flow due to T4 and GH treatment, mammary extraction of tyrosine and phenylalanine was maintained at a level (30 to 40%) similar to that observed by others (Bickerstaffe et al., 1974; Peeters et al., 1979).

Injection of T4 increased plasma phenylalanine (but not tyrosine) concentration. The mechanism for this increase is unknown.
Figure 4. Blood plasma concentration of growth hormone (a), insulin (b), thyroxine (c) and triiodothyronine (d) during T4 (---) and GH (-----) experimental periods. See legend to figure 1 for statistical details.

Cardiac Output and Mammary Blood Flow. Measurement of cardiac output in cattle has been made previously using dye-dilution (Doyle et al., 1960; Fisher and Dalton, 1961; Waldern et al., 1963) and thermodilution procedures (Skarda and Muir, 1979). Estimates by these procedures have varied from 99 to 130 ml · kg⁻¹ · min⁻¹, although it has not always been stated whether animals were lactating at the time of measurement. In goats, cardiac output increased two- to threefold around lactogenesis (Linzell, 1974), and estimates of cardiac output have been similar to cows, at 100 ml/kg.min (Chaiyabutr et al., 1980). The data obtained in our study indicated that cardiac output in untreated Jersey cows at peak lactation was 130 ml/kg.min. Further, it was shown that the thermodilution technique was suitable for cardiac output determination over extended periods. Swann-Ganz thermodilution catheters were maintained in situ for up to 4 wk before replacement or re-positioning became necessary.

Cardiac output and udder blood flow have not previously been determined together in lactating cows. The average proportion of cardiac output perfusing the udder was 15.4%, a value similar to that observed in lactating goats (Chaiyabutr et al., 1980).

With T4 treatment, the measured increase in udder blood flow accounted for 28% of the increase in cardiac output, while with GH the

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*Number of cow-days; three to four pairs of arteriovenous blood samples were taken and analyzed each day.

*Values are mean ± SE.
comparable proportion was 48%. Although no measurements of blood pressure were made, it seems reasonable to assume that the increased cardiac output was necessary to maintain blood pressure a peripheral resistance decreased in response to hormonal stimulation of "sensitive" tissues.

The increase in udder blood flow in response to T4 and GH treatment was proportionally greater (33 to 35%) than the increase in milk production (21 to 25%). In a study with GH-treated goats, increases in udder blood flow were variable and, on average, increased 21.3% relative to a 6.6% increase in milk production (Mepham et al., 1984).

The blood flow: milk yield ratios observed here (590 to 670) were higher than those reported by others measuring blood flow by EM flowmeter (Peeters et al., 1979) or continuous thermodilution (Bickerstaffe et al., 1974) but lower than obtained by antipyrine absorption (Kronfeld et al., 1968). Neither GH or T4 injections produced statistically significant changes in blood flow: milk yield ratio, although there was some indication of a rapid rise in this ratio in response to T4 injection.

**Mammary Blood Flow Determination.** Mammary blood flow has been determined previously in ruminants using EM flowmeter (Peeters et al., 1979), but continuous thermodilution has been the more popular method (Linzell, 1974) largely because of calibration difficulties associated with use of the EM flow cuff. In particular, it has been reported that temporal change in calibration can occur with EM flow cuffs following chronic implantation in vivo (Dobson et al., 1966). The present study indicates the feasibility of long-term implantation of EM flow cuffs around the external pudic artery of cattle. In vitro calibrations were verified in vivo using Fick principle methods with tyrosine and phenylalanine. However, the inherent variability of the AV difference technique and the requirement of relating "spot" AV difference measurements to daily milk protein output means that the Fick method is unsuitable for use when short-term perturbations in flow are likely to occur. Tyrosine and phenylalanine are the only group 1 amino acids for which analysis can be carried out without resorting to ion-exchange chromatography.

A prerequisite for these experiments was that feed intake remained high and stable throughout. Mammary blood flow responds relatively rapidly to nutritional status, declining to less than half of normal values with 24 h starvation (Linzell, 1974; Davis and Collier, 1985).

**Nutrient Partitioning.** It is well established that GH alters nutrient partitioning between milk and body tissue. The proportion of cardiac output delivered to the udder may have a major influence on the partition of nutrient between milk and tissue in view of the requirement that mammary tissue has for certain milk precursors. The regulation of mammary blood flow could be regarded as a major homeorhetic principle by the definition of Bauman and Currie (1980).

Data in figure 2 indicate that both GH and T4 treatment increase the proportion of cardiac output perfusing the udder, although the increase was significant only for GH treatment. This increase would permit the udder to receive a greater share of nutrient input, and was likely achieved by GH effects being relatively specific to mammary tissue (approximately half the increase in cardiac output being accounted for by the increase in udder blood flow). It is probable that the stimulation of mammary metabolism increased output of a local vasodilator, decreasing mammary vascular resistance.

However, there is no evidence that GH has a direct effect on mammary tissue, and indeed there is some evidence suggesting the contrary (McDowell and Hart, 1984; Bauman and McCutcheon, 1985).

**Blood Plasma Hormone Concentrations.** Injections of GH and T4 increased blood plasma concentrations of these hormones in samples taken 20 to 22 h later (figure 4). T3 concentrations were also elevated in response to T4 injection. GH was without effect on T4 or T3 concentrations, and conversely, T4 did not affect GH concentration, although a small rise occurred during the post-treatment period. When thyroprotein feeding increased milk production in cows, there was no change in the plasma concentration of prolactin, growth hormone or glucocorticoids (Shaw et al., 1975). Neither T4 nor GH treatment affected prolactin concentrations in blood plasma. Other data (Fronk et al., 1983) indicate that after sc injection of Holstein cows with a similar dose of GH to that used here, concentrations of GH in plasma increased to 50 ng/ml within 2 h of injection, declining essentially to control concentrations (10 ng/ml) after 24 h.

Blood insulin concentration was significantly decreased in T4-treated animals, and this was associated with hyperglycemia (Davis et
al., 1987). Blood insulin tended to increase in GH-treated cows but was not significant. Other studies have indicated that GH increased (Bines et al., 1980) or did not change Peel et al. (1981) plasma insulin content.

Literature Cited


