EFFECTS OF THYROXINE AND GROWTH HORMONE TREATMENT OF DAIRY COWS ON MAMMARY UPTAKE OF GLUCOSE, OXYGEN AND OTHER MILK FAT PRECURSORS


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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. Milk fat, lactose output, mammary uptake of glucose, oxygen and milk fat precursors were determined with each treatment. Injection of T4 increased lactose yield by 25% and fat yield 42%. The injection of GH increased fat and lactose yields by 24%. Both GH and T4 increased mammary glucose uptake by 35% and 45%, respectively, while T4 administration was associated with an increase in plasma glucose concentration from 67 to 84%. Thyroxine, but not GH, increased the ratio of mammary glucose uptake to lactose output from 1.24 to 1.58. Blood plasma acetate concentration declined following GH and T4 treatment by 17%. Mammary acetate uptake increased in response to GH injection in two of three cows but did not change with T4 injection. The injection of GH had no effect on plasma propionate concentration or mammary uptake. Thyroxine reduced plasma propionate content and mammary uptake. Neither T4 nor GH changed plasma free fatty acid concentration or mammary uptake. Thyroxine had no effect on plasma triglyceride concentration or mammary uptake, whereas GH increased mammary triglyceride uptake to the end of the experimental period. Mammary oxygen uptake was increased by GH as milk production increased. Increased mammary oxygen uptake following T4 treatment was transient. Change in mammary metabolism with T4 treatment permitted increased milk output without change in mammary oxygen consumption. Such a change may involve increased mammary utilization of pre-formed long-chain fatty acid and increased metabolism of glucose via glycolysis.

(Key Words: Somatotropin, Thyroxine, Metabolism, Milk Yield, Milk Fat, Mammary Glands.)

Introduction

Injection of growth hormone (GH) or thyroxine (T4) into dairy cows stimulates milk production by 10 to 40% (Meites, 1961; Collier et al., 1984; Davis and Bass, 1984; Bauman and McCutcheon, 1985). With T4, such increases are associated, in part, with increased milk fat and lactose content (Blaxter et al., 1949; Thomas, 1953). With GH treatment, an increase in milk fat content is usually seen only in cows in negative energy balance, and such an increase is associated with an increased proportion of longer chain fatty acids not synthesized in the udder (Bitman et al., 1984).

The manner in which hormonal treatments modify mammary metabolism may be elucidated by comparison of mammary substrate uptake from blood with milk output. A change in this ratio indicates a change in mammary metabolism (Barry, 1964). In the present study this technique was used to assess the effects of GH and T4 on mammary glucose and acetate metabolism, uptake of other fat precursors and oxygen utilization.

Materials and Methods

Experimental Design. Four lactating Jersey cows (wk 4 to 24 of lactation) were housed in...
controlled-environment chambers and fed ad libitum a diet based on corn, cotton seed hulls and soy bean meal as described in Davis et al. (1987). Experiments consisted of 16 periods during which the treatment (GH, 44 mg/d or T4, 20 mg/d) was given daily for 4 d by sc injection following 4 d of control measurements, as described in Davis et al. (1987). All cows received T4 and three cows received GH treatment. Mammary blood flow (left-udder) was determined via an electromagnetic flow cuff implanted around the left external pudic artery (Davis et al., 1987).

**Blood Sampling, Milk Sampling and Analytical Methods.** Four samples of arterial (A) and mammary venous (V) blood (subcutaneous abdominal vein) were taken each day. Arterial and venous samples were taken simultaneously while continuously recording blood flow. A composite milk sample was obtained daily from the left-udder half and yield recorded at each milking (Davis et al., 1987).

Plasma glucose, triglyceride and free fatty acids were determined colorimetrically (Itaya and Ui, 1965). Plasma acetate and propionate were determined by an ethanolic extraction procedure as described in Remesy and Demigne (1974). Milk lactose was determined as described by Marier and Boulet (1957) and fat content by the method of Babcock (Davis, 1959). Blood oxygen content was determined daily on three pairs of arterial and mammary venous blood samples using an oxygen analyzer.

Mammary oxygen uptake was predicted using a multiple regression equation generated from the data of Bickerstaffe et al. (1974):

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\text{Oxygen uptake (ml/min)} = 14.4 - .124 \text{ fat} + 1.26 \text{ protein} - .41 \text{ lactose} \quad (n = 6; R^2 = .98; \text{ residual SD} = 17; \text{ secretion rates are mg/min}).
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**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 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 traits, 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).

**Results**

**Milk Fat and Lactose Yield.** Injections of thyroxine increased milk fat content 13% from 47 to 53 g/liter (P<.01), with maximum response occurring on d 11 (figure la), but milk lactose was not affected (figure lb). Injections of GH did not alter milk fat or lactose concentration during the experimental period (figures 1a,b). Fat yield increased 42% (P<.05), from 366 to 518 g/d (half-udder), by d 11 (figure lc) with T4, whereas lactose yield increased 25% (P<.05) from 429 to 535 g/d (half-udder), with maximum yield occurring on d 11 (figure ld). The injection of GH increased fat yield (figure 1c) by 24% (P<.10; 395 to 488 g/d) and lactose yield (figure 1d) by 24% (P<.10; 425 to 527 g/d), with maximum responses by d 8.

**Mammary Glucose Uptake.** Thyroxine increased (P<.01) blood plasma glucose concentration from 67 to 53 g/liter (P<.01), with maximum response occurring on d 10 (figure 2a), whereas GH injections had no effect. Both GH and T4 injections increased mammary (half-udder) glucose uptake (figure 2c), GH by 35% (384 to 520 mg/min; P<.001) and T4 by 45% (364 to 529 mg/min; P<.05). The ratio of mammary glucose uptake to lactose output increased (P<.10) with T4 injection from 1.24 to 1.58 by d 7 and returned to control values by d 12. This ratio did not change with GH injection (figure 2d).

Mammary glucose extraction (%) declined with T4 injection as arterial glucose concentration approached a maximum (d 8), but the difference was nonsignificant (control, 20.7%; d 8, 18%). There was a nonsignificant decline in glucose extraction from 23 to 21% (d 13) during the GH experimental period.

Correlation of mammary glucose AV difference with arterial glucose concentration for control period and maximum response data

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7Lex-O-Con, Lexington Instruments, Waltham, MA.
across cows and experiments (n = 14) was significant; glucose AV difference increased by 
.16 ± .05 mg/100 ml for each 1 mg/100 ml increase in glucose concentration.

**Mammary Acetate Uptake.** Blood plasma acetate concentrations decreased following GH and T4 injections, declining 17% (P> .10) on d 12 and 17% (P< .01) on d 8 for each treatment (figure 3a). Mammary acetate uptake from plasma did not change with T4 treatment, but there was a nonsignificant increase from 201 to 245 mg/min (P> .10) by d 7 in response to GH injection (figure 3c). Mammary acetate AV difference declined (figure 3b) following T4 and GH treatment by 25% (P< .01; d 8) and 20% (P< .05; d 12), respectively. These reductions were associated with the decline in arterial plasma acetate concentration (figure 3a). Correlation of mammary acetate AV difference with arterial acetate concentration for control period and maximum response data across cows and experiments (n = 14) was significant, with acetate AV difference increasing by .69 ± .10 mg/100 ml for each 1 mg/100 ml change in arterial acetate concentration.

**Mammary Propionate Uptake.** The injection of GH had no effect on arterial plasma propionate concentration (figure 4a), AV difference (figure 4b) or extraction percent (mean extraction rate 40%). Mammary propionate uptake increased 39%, from 4.9 to 6.8 mg/min by d 8 (figure 4c), but this change was not statistically significant. The injection of T4 reduced arterial plasma propionate concentration from .58 to 139 mg% (figure 4a) by d 9 (P< .01). This 33% decrease was associated with a reduction in AV difference (figure 4b) from .24 to .14 mg% (−42%; P< .05) and percent extraction from 40 to 34% (−15%; P< .10). Mammary propionate uptake declined from 6.6 to 4.9 mg/min (−26%; P< .10) by d 10 (figure 4c). Correlation of mammary propionate AV difference with arterial propionate concentration for control period and maximum response data across cows and experiments (n = 14) was significant, with propionate AV difference increasing by .48 mg/100 ml for each 1 mg/100 ml change in plasma propionate concentration.

An inverse relationship between plasma propionate concentrations and milk fat output has been noted previously (Williams and Elliot, 1980). In the present study blood propionate concentrations, mammary extraction and uptake were similar to those obtained by others.
Figure 2. Arterial plasma glucose concentration (a), mammary glucose AV difference (b), mammary glucose uptake (c) and ratio of mammary glucose uptake to lactose output (d) during T4 (-----) and GH (- - -) experimental periods. See legend to figure 1 for statistical details.

Figure 3. Arterial plasma acetate concentration (a), mammary acetate AV difference (b), mammary acetate uptake (c) and ratio of mammary acetate uptake to milk fat output 3d during the T4 (-----) and GH (- - -) experimental periods. See legend figure 1 for statistical details.
Mammary Triglyceride and Free Fatty Acid Uptake. There was no detectable effect of T4 injection on plasma triglyceride concentration (figure 5a; mean range 13.5 to 13.7 mg/100 ml) or mammary extraction (mean 45%). There was an increase (P<.001) in mammary triglyceride uptake from 336 to 445 mg/min beginning within 24 h of the first GH injection (figure 5b). This increased uptake was sustained until the end of the experimental period. The ratio of mammary triglyceride uptake: milk fat output followed a similar pattern, increasing from 1.03 to 1.33 (P<.10).

Mean plasma FFA concentrations ranged from 366 to 445 μeq/liter during T4 and GH experimental periods, respectively (figure 5c). There was no significant change in mammary uptake of FFA during both experimental periods (figure 5d). Similarly, there was no significant change in percent mammary extractions of free fatty acids during both treatments. However, mammary venous FFA include FFA released by triglyceride hydrolysis, so no conclusions can be drawn as to the relative contributions of TG and FFA to mammary fat synthesis.

Mammary Oxygen Uptake. Arterial concentrations of oxygen were not altered by either treatment (figure 6a). Mammary oxygen AV difference declined with T4 (P<.10) but not GH treatment (figure 6b). Mammary oxygen extraction varied between 30 to 40%, but differences between control and treatment periods were not significant.

Mammary oxygen uptake (half-udder) increased from 161 to 180 ml/min (P<.05) within 2 d of the start of the T4 injections (figure 6c). Predicted oxygen uptake increased (figure 6d) from 172 to 201 ml/min (P<.10) over the same period, and both predicted and measured uptakes did not differ from control values by d 10, a time when milk lactose, fat and protein output were still elevated (figures 1c,d; Davis et al., 1987).

The response pattern of mammary oxygen uptake to GH injection was slower than the response to T4, and a 37% increase in oxygen consumption (from 145 to 198 ml/min) was observed by d 11. Oxygen uptake had returned to control levels by d 16 (figure 6c). The pattern of response of predicted oxygen uptake followed measured uptake closely (figure 6d), increasing 31% from 167 to 218 ml/min, by d 11 (P<.10). Overall, predicted mammary oxygen uptake was closely correlated with measured oxygen uptake during control and treatment periods.

(Bickerstaffe et al., 1974; Williams and Elliot, 1980). Treatment by T4 reduced blood propionate concentrations and mammary uptake during the period that milk fat output was increasing (figure 1c); however, there is no evidence that propionate can have a direct effect on mammary tissue to influence milk fat synthesis (Croom et al., 1981a,b).

Mammary Triglyceride and Free Fatty Acid Uptake. There was no detectable effect of T4 injection on plasma triglyceride concentration (figure 5a; range 31 to 43 mg/100 ml), mammary AV difference (mean range 6.3 to 8.3 mg/100 ml), percent extraction (mean range 23.0 to 35.0%) mammary uptake (figure 5b; 216 to 233 mg/min), or the ratio of triglyceride uptake: milk fat output (.58 to .84). Similarly, GH injection had no effect on arterial plasma triglyceride concentration (figure 5a; mean range 13.5 to 13.7 mg/100 ml) or mammary extraction (mean 45%). There was an increase (P<.001) in mammary triglyceride uptake from 336 to 445 mg/min beginning within 24 h of the first GH injection (figure 5b). This increased uptake was sustained until the end of the experimental period. The ratio of mammary triglyceride uptake: milk fat output followed a similar pattern, increasing from 1.03 to 1.33 (P<.10).

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Figure 5. Arterial plasma triglyceride concentration (a), mammary triglyceride uptake (b), arterial plasma free fatty acid concentration (c), and mammary free fatty acid uptake (d) during T4 (-----) and GH (----) experimental periods. See legend to figure 1 for statistical details.

Figure 6. Arterial blood content (a), mammary oxygen AV difference (b), mammary oxygen uptake (c) and predicated mammary oxygen uptake (d) during T4 (-----) and GH (----) experimental periods. Predicated mammary oxygen uptake was calculated using the equation given in text. See legend of figure 1 for statistical details.
uptake (P<.001). Comparing control period data for all cows, the mean measured oxygen uptake was .92 ± .05 of predicted oxygen uptake, and this ratio did not differ significantly from unity.

Discussion

Milk Yield and Composition. Peak production of milk lactose and fat occurred between d 10 and 12 with T4 treatment, whereas GH injections resulted in peak production of these components by d 8. These results were achieved during the time of peak milk yield response to both treatments (Davis et al., 1987).

Most of the increase in lactose and fat output was associated with the increase in milk yield with both treatments (23%). However, with T4 treatment, milk fat output increased by 42% because milk fat content increased by 14% (figure 1c). Such increases have often, but not always, been observed in response to T4 injection or thyroprotein feeding of cattle (Thomas, 1953). Injections of GH have usually increased milk fat content only in cows initially in negative energy balance (Bitman et al., 1984). In previous studies small increases (.2 to .3%) in milk lactose content have occasionally been observed in response to T4 treatment (Oshima et al., 1980; S. R. Davis, P. D. Gluckman and I. C. Hart, unpublished data). In this study, neither T4 nor GH increased milk lactose concentrations.

It is unlikely that a 4-d period was sufficient to elicit maximum production response with either treatment. Machlin (1973) noted that milk production continued to increase for up to 10 d after GH injections. Some of the changes that occurred in milk composition in the present study may have been transient, primary responses to the brief period of stimulation. For example, Smith and Dastur (1940) showed that changes in milk fat composition (decreased Reichert and Meiss value and increased iodine number) were maximal in cattle by d 3 of GH treatment but reverted to control values over the next few days. In short-term studies such as the present, the adjustment of whole animal metabolism to the increased metabolic drain may have been incomplete.

Mammary Glucose Metabolism. There were two major differences in the effects of T4 and GH on mammary glucose availability and metabolism. First, blood plasma glucose concentrations were elevated by T4 but not GH treatment (figure 2a). Second, T4, but not GH, treatment increased the ratio glucose uptake: lactose output (figure 2d).

There are three pathways of glucose metabolism in mammary tissue: lactose synthesis, glycolysis and the pentose phosphate shunt. Lactose synthesis is quantitatively the major route of glucose utilization. In a perfused udder preparation in vitro, lactose synthesis, glycolysis and the pentose phosphate shunt accounted for 62, 8 and 30%, respectively, of mammary glucose uptake (Wood et al., 1965). In vivo studies have indicated that the ratio of mammary glucose uptake to lactose output in cattle is 1.2 to 1.3 (Bickerstaffe et al., 1974; Peeters et al., 1979), which is similar to those ratios determined in this study during control periods (figure 2e).

Depression of milk secretion in cows has been noted when plasma glucose concentrations declined below 60 mg% following insulin administration (Kronfeld et al., 1963). Rulquin (1981) reported that there was no correlation between mammary glucose AV difference and arterial concentration in a study with cows, although Linzell (1960) showed that increasing arterial glucose concentration was associated with increasing mammary glucose AV difference. Such a relationship was apparent when separate studies with cows were compared in a recent review (Davis and Collier, 1985); these studies support the findings of the present study.

Elevation of blood glucose concentration with T4 injections was associated with a depression in plasma insulin concentration (Davis et al., 1987) and an increase in milk and milk fat yield. In contrast, hyperglycemia induced by administration of dexamethasone has been associated with depression of milk secretion (Warner and Johnson, 1983). In previous studies, hyperglycemia arising from abomasal or intravenous glucose infusion was associated with small increases in milk production and depression of milk fat secretion (Frobish and Davis, 1977; Chaiyabutr et al., 1983).

It is unlikely that the galactopoietic effects of T4 were "driven" by hyperglycemia; rather, the increased mammary glucose uptake (relative to lactose output) reflected an increase in mammary glucose requirement and metabolism via glycolytic and pentose phosphate pathways. Increased activity of the latter pathway may be required to generate NADPH for fatty acid synthesis.
The ratio of glucose uptake to lactose output increased from 1.23 to 1.58 by d 6 and 7. This increase approached statistical significance (P< .10) and was observed in all T4-treated cows but delayed in one animal.

**Mammary Oxygen Uptake.** Mammary oxygen uptake is closely linked to the activity of the tricarboxylic acid cycle and the production of ATP by oxidative phosphorylation. The synthesis of lactose, protein, and fat requires the utilization of ATP and/or GTP. The production of lactose requires 6 mmol ATP/g synthesized; protein requires 15 mmol ATP/g, and fat synthesis requires 11 mmol ATP for triglyceride assembly and an additional 27 mmol ATP/g fatty acid synthesized (Smith et al., 1983).

By d 10 following GH injection, lactose, protein, and fat output had increased by 95, 74, and 85 g/d, respectively, whereas mammary oxygen consumption had increased 35% from 145 to 195 ml/min (P<.10). In contrast, in the T4 experiment, lactose, protein, and fat output had increased by 106, 50, and 150 g/d, respectively, but mammary oxygen consumption did not differ from the control period. In confirmation of the direct measurements, the pattern of predicted oxygen uptake and measured oxygen uptake were closely matched in both GH and T4 experiments.

It is worth considering the possible mechanisms by which mammary tissue might increase milk synthesis (and ATP utilization) without altering oxygen consumption. There are three possibilities: 1) tighter coupling of phosphorylation to oxidation in the electron transport chain; 2) increasing supply of ATP from satellite pathways including glycolysis, glutamate oxidation and lactate oxidation; and 3) increased mammary utilization of preformed long-chain fatty acids, decreasing mammary ATP requirements for fatty acid synthesis.

It can be calculated that about 3.5 mol ATP would be required to support the increased milk output from T4 treatment. The increase in mammary glucose uptake, during T4 injections, above that required for lactose synthesis was 104 g/d, sufficient to provide about 1 mol ATP via glycolysis. It is possible that further ATP might be generated via glycolysis if pentose phosphate shunt activity were reduced in response to a decrease in NADPH requirements for mammary fatty acid synthesis.

In ruminants, acetate is used for the synthesis of milk fatty acids up to and including part of C16:0. Bickerstaffe (1971) noted that mammary C16:0 uptake was sufficient to account for 25 to 50% of C16:0 output, the latter constituting 26% by weight of milk fat. Substitution of C16:0 from blood triglyceride and free fatty acids for mammary synthesized C16:0 could significantly reduce mammary ATP (and NADPH) utilization in fatty acid synthesis. For example, reducing the proportion of mammary synthesized C16:0 from 50 to 25% of total milk C16:0 output would release about 1 mol of ATP for alternative uses. The failure of T4 injections to change mammary acetate uptake in spite of increased milk fat synthesis support this mechanism. Furthermore, such a shift in mammary metabolism may occur without large changes in milk fat composition. In lactating ewes, where the ratio of mammary glucose uptake to lactose output is 2.0 (Davis and Bickerstaffe, 1978), 40% of glucose uptake appears in mammary venous blood as lactate (Mercer et al., 1980).

In contrast to T4, the extra ATP required for milk synthesis following GH stimulation appears to be derived from oxidation phosphorylation and does not require alteration of the normal energy producing processes in the cell. Such differences between T4 and GH responses may explain why their effects on milk synthesis are additive (Young, 1947; Meites, 1961).

**Mammary Fat Metabolism.** Precursors of milk fat include plasma acetate, free fatty acids and triglyceride fatty acids. Only uptake of triglyceride fatty acids necessitates enzymatic hydrolysis via lipoprotein lipase (LPL). The concentrations of triglyceride in plasma were within the ranges reported by others for dairy cows (Davis and Collier, 1985). Mammary percent extraction and uptake were also comparable to reported values. Certainly GH increased the mammary uptake of triglyceride, and this was sustained until d 16, even through the period when milk fat yield was in decline. Triglyceride uptake at the levels measured was sufficient to account for most milk fat output and certainly all the milk fat fraction known to be derived from plasma triglyceride (50% by weight). Any free fatty acid (FFA) released from triglyceride in excess of milk fat synthesis requirements could then be released to venous circulation. In spite of a 42% increase in milk fat yield, mammary triglyceride uptake apparently did not change in response to T4 treatment. More conclusive statements must await the measurement of free fatty acid uptake using
radiolabelled tracers and the response of mammary LPL activity to hormone treatment.

Plasma acetate content was much less variable, and mammary acetate uptake did not change in response to T4 injection. However, changes in mammary glucose metabolism may have decreased acetate oxidation in order to spare it for use in milk fat synthesis. The treatment of GH increased mammary acetate uptake by 22%, although this increase was not significant. Bitman et al. (1984) noted that with cows in negative energy balance, GH injection increased milk fat yield by 42%. Milk fat composition was altered in favor of long-chain fatty acids by 6% at the expense of fatty acids of 14 carbons or less. These changes were associated with an increase in the proportion of free fatty acids in plasma. In the present study the failure of the arterial FFA to increase with hormone treatment may have been due to the fact that the cows used were most likely in a positive energy balance.

There is a lack of data describing the effects of T4 on milk composition and blood lipids. Loginov (1975) indicated that there were increased proportions of C18:0 and C18:1 in milk fat of cows following T4 treatment whereas, in contrast, Azimov et al. (1964) have shown that there was a uniform increase in secretion of both short- and long-chain fatty acids in milk. Smith and Dastur (1940) noted that T4 evoked extremely small changes in milk fat composition in cows and little change in triglyceride fatty acids in blood plasma. The findings in the present study are consistent with increased precursor availability for fatty acids of all chain lengths for milk fat synthesis during both GH and T4 treatments.

**Literature Cited**


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