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Effect of Somatotropin on Thyroid Hormones and Cytokines in Lactating Dairy Cows During Ad Libitum and Restricted Feed Intake[1]

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ABSTRACT

Twelve Holsteins in first lactation were used to investigate the relationship between energy balance and effects of bovine somatotropin (bST) on thyroid hormone metabolism and cytokine concentrations in serum. Six cows were fed for ad libitum intake and six cows were feed restricted to induce negative energy balance during two treatment periods of 6 d. During treatment periods, cows were administered vehicle or 40 mg of bST/d according to a crossover design. Between treatment periods was a 15-d recovery period, during which all cows were fed ad libitum. Cows that were fed ad libitum remained in positive energy balance during control and bST treatments, whereas cows that were fed for restricted intake were in negative energy balance during control and bST treatment periods. In both dietary groups, bST decreased energy balance. Milk production and the fat percentage of milk increased during bST treatment in both dietary groups. Fat-corrected milk yield was increased 13% by bST treatment. Serum concentrations of IGF-I did not differ between dietary groups but were greater during bST than control periods. Serum thyroxine concentration was decreased by bST treatment. Serum triiodothyronine and reverse-triiodothyronine were not altered by hormone treatment, but circulating concentrations of thyroid hormones were apparently reduced by dietary restriction. Neither hepatic nor mammary thyroxine 5′-deiodinase was affected by bST treatment. Plasma concentration of tumor necrosis factor-α, a potential regulator of thyroxine 5′-deiodinase, was not affected by bST treatment. Short-term treatment with bST did not influence thyroid hormone metabolism in lactating cows in positive or negative energy balance.
(**Key words:** thyroxine- 5′-deiodinase, energy balance, somatotropin, cytokine)

Abbreviation key: $\mathbf{rT_3}$ = reverse-triiodothyronine, $\mathbf{T_3}$ = triiodothyronine, $\mathbf{T_4}$ = thyroxine, **TNF** = tumor necrosis factor-α, **5′D** = thyroxine 5′-deiodinase.

INTRODUCTION

Thyroid hormones are important regulators of mammary gland function. In the absence of thyroid hormones, growth and differentiation of the mammary epithelium are reduced (Vonderhaar and Greco, 1979). Furthermore, thyroid hormones are galactopoietic, and administration of bST to lactating dairy cows was shown to alter metabolism of thyroid hormones in a tissue-specific fashion that maintains the euthyroid status of the mammary gland in the presence of a systemic hypothyroid state. This is consistent with the ability of bST to establish a metabolic priority for the mammary gland (Capuco et al., 1989; Kahl et al., 1995). Data from mice indicate that thyroid hormones are necessary to obtain a milk production increase in response to administration of galactopoietic hormones (Capuco et al., 1999).

Although thyroxine ($\mathbf{T_4}$) is the predominant thyroid hormone in the circulation, it has little inherent biological activity and it is commonly viewed as a prohormone. The metabolically active thyroid hormone, triiodothyronine ($\mathbf{T_3}$), is produced by enzymatic 5′-deiodination of T_4 within the thyroid and in extrathyroidal tissues (Leonard and Visser, 1986). Conversely, 5-deiodination of T_4 generates the inactive thyroid hormone metabolite, reverse-T_3 ($\mathbf{rT_3}$). The extrathyroidal activity of thyroxine 5′-deiodinase (**5′D**) is an important control point for regulating the thyroid status of animal tissues in various physiological and pathological situations (Cho-

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pra et al., 1978; Kohrle, 1994; Larsen, 1982; Leonard and Visser, 1986; Wartofsky and Burman, 1982). In addition to bST, cytokines are potential regulators of 5′D (Ongphiphadhanakul et al., 1994; Pekary et al., 1994; Tang et al., 1995). Tumor necrosis factor-α (**TNF**) decreases 5′D activity in peripheral tissues during starvation and low T_3 syndrome (Pang et al., 1989) and may play a regulatory role in other physiological states (Bartalena et al., 1998).

Alterations in production of cytokines and IGF-I in response to bST administration may account for some of the observed effects of bST on immune function and endocrine status (Burvenich et al., 1999; Hoeben et al., 1999; Vandeputte-Van Messom and Burvenich, 1993). Additionally, the animal's energy balance may influence the effect of bST on thyroid hormones and influences immune function (Elsasser et al., 1997; Powanda, 1980).

The objective of the present study was to determine the effect of restricted feed intake on thyroid hormone metabolism, IGF-I and responses of TNF to bST treatment of lactating dairy cows.

MATERIALS AND METHODS

Cows and Experimental Design

Twelve primiparous Holstein cows (132 ± 8 DIM) were paired based on milk production and randomly assigned to be fed ad libitum or a restricted intake (80% of their ad libitum intake) for two 6-d periods. Three of the six cows in each dietary group were randomly assigned to receive subcutaneous injections of bST (40 mg of sometribove/d at 1000 to 1100 h) for the first period and excipient for the second period. The other three cows received excipient for the first period and bST for the second period. Thus, six cows were fed ad libitum and six were fed at restricted intake, but all cows underwent a bST and a control period. There was a 15-d ad libitum feeding period before initiation of the first treatment period and between first and second treatment periods. Sometribove was kindly provided by Monsanto Co. (St. Louis, MO).

A sterile polyvinyl cannula was inserted into a jugular vein of each cow on day 5 of each treatment period. The following day, 12 hourly jugular venous blood samples were collected, beginning at 0830 h and ending at 1930 h, after which the cannula was removed. Serum was prepared and frozen at –80°C for subsequent analyses.

Mammary and liver biopsies for determination of 5′D activity were obtained on the last day (day 6) of each treatment period, approximately 22 to 24 h after the previous bST injection. Core mammary biopsies (≈1 g) were taken from alternate rear quarters as previously described (Farr et al., 1996). Liver samples were obtained with a biopsy needle. In brief, cows were tranquilized with xylazine (10 mg, i.v.) and locally anesthetized with lidocaine. A sterile 13 gauge × 2 inch, thin-wall stainless steel needle then was introduced through the intercostal space between the third and second rib and into the abdomen. A 14-gauge × 6-inch Tru-cut biopsy needle (Baxter Healthcare Corp., Deerfield, IL) was then inserted through the 13-gauge needle to obtain hepatic tissue samples. The biopsy needle could be inserted through the 13-gauge needle several times to obtain sufficient tissue (≈60 mg). Tissues were frozen in liquid nitrogen and stored at –80°C until assay.

Cows were housed in tie stalls with 12 h of light/d and were milked twice daily. Cows were weighed twice weekly and fed daily at 0900 h. Feed consumption was monitored by weighing the feed offered and the orts. Samples of the TMR were taken throughout the study and composition determined (Forage Testing Laboratory, Ithaca, NY). All cows consumed the same diet. Ingredients and composition of the TMR are summarized in Tables 1 and 2. Milk yield was recorded daily and milk samples were collected at each milking for compositional analysis. Milk composition was determined by the Environmental System Service Ltd. (College Park, MD) by using a Foss Milko-Scan (model 104 with the A filter; A/S Foss Electric, Hillerød, Denmark). Average milk yield and composition for the last 5 d

Table 1. Ingredients and average composition of diet.

	(% of DM)
Diet composition	
Alfalfa silage	18.3
Corn silage	29.7
Supplement, minerals, vitamins[1]	46.5
Cotton seed	5.5
Forage analysis	
Crude protein	17.5
Available protein	16.2
Soluble protein	41.7
Acid detergent fiber	22.8
Neutral detergent fiber	30.7
NE_L mcal/kg	1.63
Calcium	0.83
Phosphorus	0.20
Magnesium	0.30
Potassium	1.28
Sodium	0.42
Sulfur	0.19
	(ppm)
Iron	265
Zinc	65
Copper	18
Manganese	60
Molybdenum	1.6

[1]Composition of the concentrate supplement is provided in Table 2.

Table 2. Composition of supplement.

Ingredients	%
Soybean meal	13.74
Corn gluten meal	2.85
Dry molasses	2.27
Protein blend	1.85
Limestone	1.75
Sodium bicarbonate	1.5
Megalac	1.0
Dicalcium phosphate	0.9
Sodium chloride	0.85
Magnesium oxide	0.52
Urea	0.37
Yeast culture extract	0.37
Selenium, 0.02%	0.1
Trace minerals[1]	0.07
Roasted soybeans	7.55
Ground corn	64.22
Vitamin premix	0.12
Vitamin A, 10,009 IU/g	
Vitamin D, 2205 IU/g	
Vitamin E, 1.10 IU/g	

[1]Ca, 6.7 to 8.0%; Zn, 6.7%; Mn, 10.67%; Cu, 2.67%; Fe, 0.933%; I, 0.403%; Co, 0.108%.

(10 samples) of each treatment period was used for statistical analysis.

Use of animals for this investigation was approved by the Beltsville Agricultural Research Center's Animal Care and Use Committee.

Energy Balance Calculations

Daily energy balance was calculated for individual cows by using estimates of net energy intake and energy requirements for maintenance and milk production (NRC, 2001). Milk energy output was calculated based on measured daily yields of fat, protein, and lactose and calculated by using equations for individual milk components as described by NRC (2001). Maintenance energy requirements expressed on any given day, expressed in NE_L equivalents, were based on the average of two consecutive previous BW measurements. These were used to calculate metabolic weight and subsequent NE_L required for maintenance according to NRC (2001).

Feed energy intake was calculated by using daily DMI and estimated diet NE_L concentrations from feed analyses (Forage Testing Laboratory) of total mixed diets fed during the experiment. These feed energy values were determined for three times maintenance intake (National Research Council, 1989), because the fat and lignin contents were not determined and are required for continuous adjustment of feed energy values as described in NRC (2001). Net energy for lactation balance was calculated as the difference between NE_L intake and NE_L requirements for maintenance and milk production. Net tissue energy balance was calculated from NE_L balance by using a 0.82 efficiency for use of mobilized tissue energy for milk energy (NRC, 2001). When cows were in positive NE_L balance, NE_L was converted to tissue energy balance by using a 0.75 efficiency of use of dietary ME compared with 0.64 for milk energy. Thus, 1 Mcal of positive NE_L balance would be equivalent to 1.17 Mcal of tissue energy balance (NRC, 2001).

Serum Hormones and Metabolites

Serum concentrations of T_4 and T_3 (ICN Biomedical Inc., Carson, CA) were determined in duplicate on hourly samples 1, 3, 5, 7, 9, and 11 of each treatment period, using commercially available radioimmunoassay kits. Serum concentrations of rT_3 (PolyMedCo, Cortland Manor, NY) were determined in duplicate on hourly samples 1 and 11 of each treatment period. Serum concentrations of IGF-I were determined in duplicate on hourly samples 2 and 12 of each treatment period, as described previously (Elsasser et al., 1989). Serum concentrations of growth hormone were determined (Capuco et al., 1989) in duplicate on the 0830 h sample (coinciding with the average time when biopsies were taken on the following day) for each treatment period. Serum concentrations of TNF were determined in triplicate on hourly samples 2, 6, 10, and 12 of each treatment period, as described previously (Kenison et al., 1990).

Serum concentrations of neutral lipids were determined by quantitative TLC (Bitman et al., 1992) by using blood obtained by cocygeal venipuncture at the time of tissue biopsy.

5′D Activity

Liver and mammary tissues were homogenized (1:10, wt/vol) in HEPES buffer (0.01 *M* HEPES, pH 7.0, containing 0.25 *M* sucrose and 5 m*M* EDTA). Outer ring deiodination activity was determined by quantifying the $^{125}I^-$ released from 3,3′,5′-[^{125}I]-T_3 (rT_3) on a portion of the tissue homogenates as previously described (Jack et al., 1994). Homogenate protein concentration was determined by the bicinchoninic acid assay (Pierce Chemical Company, Rockford, IL), using BSA as standard.

Statistical Analysis

Data were analyzed by using the mixed procedure of SAS (version 8; SAS Inst. Inc., Cary, NC) as a crossover design.

$$Y_{ijklm} = \mu + EB_{ijklm} * \beta + seq_i + trt_j + cow_{ik} + per_l + \varepsilon_{ijklm}$$

where:
μ is an overall mean,
EB_{ijklm} is the energy balance on the day of observation and was included for hormone variables, but was excluded for production variables and NEFA,
β = covariate for EB,
seq_i is the fixed effect of sequence of treatments (i = 1,2,3,4),
1 = bST treatment first, ad libitum intake,
2 = control treatment first, ad libitum intake,
3 = bST treatment first, restricted intake,
4 = control treatment first, restricted intake,
trt_i was the fixed treatment effect (i = 1,2,3,4),
1 = bST, ad libitum intake,
2 = control, ad libitum intake,
3 = bST, restricted intake,
4 = control, restricted intake,
cow_{ik} was the random cow effect,
per_l was the random period effect, and
ε_{ijklm} was the random residual.

To facilitate estimation of intake effect, analyses for some traits were possible by using the adjustment period as a treatment. Without this addition, differences between diet effects were not estimable by using the described model and data structure (because cows were kept in ad libitum or restricted groups for both treatment periods). The adjustment period was considered treatment zero and provided a connection between the two diet groups. Note that the recovery period was not included in any analyses.

Point estimates were calculated for each treatment by using an "estimate" statement that included the mean, appropriate treatment and equal parts of the appropriate sequence effects. For example, the estimate for treatment 1 mean was calculated as $\hat{\mu} + \hat{trt}_1 + \frac{1}{2}(\hat{seq}_1 + \hat{seq}_2)$. When estimating these effects for models that included energy balance as a covariate, the solution for the covariate was weighted by the mean energy balance for that treatment. Analyses of production traits (Table 3) and serum NEFA did not include EB (energy balance) as a covariate. Analyses of hormone data and 5′D activity included EB as covariate.

For traits that were measured repeatedly (milk yield, milk composition, milk energy, DMI, energy balance), a first-order autoregression among residual effects was assumed for cows in each treatment period (final 5 d of the treatment period). Because all cows were fed ad libitum immediately after biopsy (22 to 24 h after injection of bST or vehicle), values from the previous day were used for EB covariate of those parameters evaluated at biopsy (liver 5′D and mammary 5′D).

RESULTS

Cows that were provided TMR at 80% of their ad libitum intake rapidly entered a period of negative energy balance for the duration of restricted intake ($P < 0.0001$; Table 3, Figure 1). Treatment with bST decreased energy balance in both dietary groups ($P = 0.0006$). Energy balance reached a nadir within the first 3 d of restricted feeding (Figure 1). When feed-restricted cows were treated with bST, energy balance was maintained at this low state; but without bST, energy balance became less negative after the second day of restricted intake. This improvement in energy balance coincided with a numerical decrease in milk production (Figure 1), although milk production did not differ ($P = 0.304$) between restricted and ad libitum feeding periods. Restricted feeding caused a decline in BW ($P < 0.05$, data not shown), with recovery occurring between treatment periods. Mean BW for ad libitum fed cows were 529 and 540 kg on day 1 of treatment periods 1 and 2, respectively ($P = 0.038$, paired t-test). Mean BW for restricted cows were 556 and 555 kg on day 1 of treatment periods 1 and 2, respectively ($P = 0.073$, paired t-test).

Milk fat increased and SNF and protein decreased in response to bST treatment in both intake groups ($P < 0.0001$). Differences in FCM due to bST were identical in the ad libitum and restricted feeding groups. Milk lactose concentration was unaffected by bST treatment but was decreased by restricted feeding ($P = 0.015$). Consistent with induction of negative energy balance, dietary restriction seemingly enhanced mobilization of lipid stores as evidenced by a 2.8-fold greater concentration of serum NEFA for control cows fed restricted versus ad libitum intake (Figure 2). Additionally, serum NEFA were increased by administration of bST to cows in both intake groups ($P = 0.008$). Concentrations of other neutral lipids in serum (cholesterol, cholesteryl esters, and triglycerides) were not affected by dietary restriction or bST treatment ($P > 0.1$, data not shown).

Concentrations of serum IGF-I were increased ($P < 0.0001$) by bST treatment in both intake groups, averaging 87 ng/ml during control and 214 ng/ml during bST treatment periods (Figure 2). Serum concentrations of T_4, T_3, and rT_3 were not altered by bST administration, although restriction of dietary intake seemingly decreased serum concentrations of the three iodothyronines (Figure 3, statistical significance not testable by model). Approximately 24 h after bST administration, serum concentrations of somatotropin averaged 8.22 ± 0.67 compared with 2.49 ± 0.26 ng/ml after administration of vehicle.

Serum concentrations of TNF were not influenced by bST administration ($P = 0.895$, Figure 4), nor was activity of hepatic ($P = 0.232$) or mammary ($P = 0.072$) 5′D affected by bST or feed intake (Figure 5). In the latter case, the trend toward reduced mammary 5′D with bST treatment was confounded by an apparent

Table 3. Effect of bST and feed intake on production variables.

		Ad libitum intake		Restricted intake[1]			Probability[2]			
	Adjust[3]	Control	bST	Control	bST	SE	bST	Intake	bST × I	Seq
4% FCM, kg/d	25.0	25.9	29.3	24.2	27.6	1.11	<0.0001	0.304	0.961	0.800
Fat, %	3.48	3.55	3.80	3.74	3.94	0.115	<0.0001	0.404	0.916	0.878
SNF, %	8.65	8.85	8.54	8.88	8.64	0.169	<0.0001	0.236	0.593	0.407
Protein, %	3.06	3.13	2.84	3.22	2.97	0.124	<0.0001	0.223	0.628	0.175
Lactose, %	4.89	5.00	4.98	4.92	4.93	0.053	0.960	0.015	0.343	0.657
DMI, kg/d	19.1	20.6	20.1	15.6	15.8	0.70	0.592	<0.0001	0.402	0.353
NE_L intake, mcal/d	30.7	33.8	32.9	25.7	25.8	1.27	0.568	<0.0001	0.412	0.357
Maintenance energy required, mcal/d	8.93	8.88	9.00	9.08	9.16	0.17	0.016	0.161	0.659	0.155
Milk energy, mcal/d	18.5	19.3	21.3	18.0	20.1	0.81	<0.0001	0.163	0.791	0.788
NE_L balance, mcal/d	3.3	5.5	2.8	−1.5	−3.4	1.32	0.0007	<0.0001	0.5629	0.720
Tissue energy balance, mcal/d	3.8	6.5	3.2	−1.8	−4.2	1.67	0.0006	<0.0001	0.583	0.724

[1]Feed offered was restricted to 80% of the cow's ad libitum intake.

[2]Probability for main effects, interaction of bST × Intake (bST × I), and sequence of hormone treatment (Seq).

[3]Adjust = Adjustment period; bST × I = interaction effects of bST and dietary intake; Seq = treatment sequence effects; SE = pooled standard error (largest SE is reported).

effect of treatment sequence ($P = 0.020$). The sequence effect was, in turn, due to an ostensible randomization problem. The greatest 5′D activity (liver and mammary) was observed in cows on treatment 3 (restricted + bST) and the lowest 5′D activity was observed in cows on treatment 4 (restricted + control).

With the exception of mammary 5′D activity, described above, there was no effect of treatment sequence ($P \geq 0.175$) for any of the measured parameters. Thus, the recovery period was sufficient for parameters to return to baseline levels between treatments and for treatment response to be unaffected by previous treatment.

DISCUSSION

This experiment examined the influence of energy balance on metabolic adaptations of lactating dairy cows treated with bST. Of particular interest was the relationship between the somatotropic axis and thyroid hormone metabolism.

As expected, milk yield was increased by bST in cows that were in positive energy balance and in those that were induced into negative energy balance by restricting feed availability to 80% of ad libitum intake. Restriction of feed consumption increased lipolysis of body fat depots as evidenced by increased concentrations of NEFA in serum. Concentrations of NEFA in serum were also increased by bST administration. This is in agreement with previous investigations of effects of bST during the early phase of treatment, when feed consumption had not increased to balance energy output for increased milk synthesis (Bitman et al., 1984; Tyrrell et al., 1988). Serum NEFA concentrations suggest that cows were in similar energy balance during restricted feeding periods (regardless of hormone treatment) and bST treatment periods (regardless of dietary intake). However, energy balance differed between all treatments. Tissue energy balance for ad libitum, control (6.5 mcal/d) > ad libitum, bST (3.2 mcal/d) > restricted intake, control (−1.8 mcal/d) > restricted intake, bST (−4.2 mcal/d). Others have demonstrated that serum NEFA is not a good prognosticator of energy balance, particularly in the present circumstance where the stress of venipuncture may have produced a greater lipolytic response in bST-treated than in control cows, because bST heightens sensitivity of adipocytes to catecholamines (Boisclair et al., 1997). In no case was there an uncoupling of IGF-I generation in response to bST, as can occur during severe negative energy balance (Gluckman et al., 1987; McGuire et al., 1992).

In the present study, serum concentrations of T_4 were increased 12% by bST administration, whereas concentrations of T_3 and rT_3 were unaffected. These results suggest that bST was without effect on hepatic 5′D, a major regulator of T_3 in the circulation. Similarly, negative energy balance, induced by restricted feeding, appeared to decrease concentrations of these thyroid hormones in the circulation by mechanisms that did not elicit an effect on hepatic 5′D. Indeed, determination of 5′D in liver and mammary biopsy samples indicated that activity of 5′D was unaffected in either tissue. These data do not support our hypothesis (Capuco et al., 1989, 1999; Kahl et al., 1995) that organ-specific changes in thyroid hormone metabolism play a role in eliciting a galactopoietic response to bST in dairy cows. However, 5′D probably has a rapid turnover rate similar to other regulatory enzymes. Because tissue biopsies were obtained immediately after milking and before administration of bST, 5′D activity may have been at its nadir, which may have precluded observing treatment effects. In previous experiments, tissue was obtained

several hours after bST injection and the a.m. milking of twice daily milking (Capuco et al., 1989), or during continuous bST infusion and several hours after the first of three times daily milkings (Kahl et al., 1995). Indeed, one-half of 5′D activity in rat mammary gland is lost 12 h after cessation of suckling and may be rapidly increased by restoration of suckling via mechanisms that include pre- and posttranslational effects of neuroendocrine factors (Aceves et al., 1999). A rapid turnover rate for 5′D may explain the lack of a bST effect on 5′D in mammary or liver tissues. Although this may be a confounding factor, the lack of a bST effect on hepatic 5′D is consistent with its lack of effect on concentrations of T_3 in serum. The reduction in circulating concentrations of thyroid hormones during negative energy balance is consistent with a decrease in hormone secretion rather than metabolism.

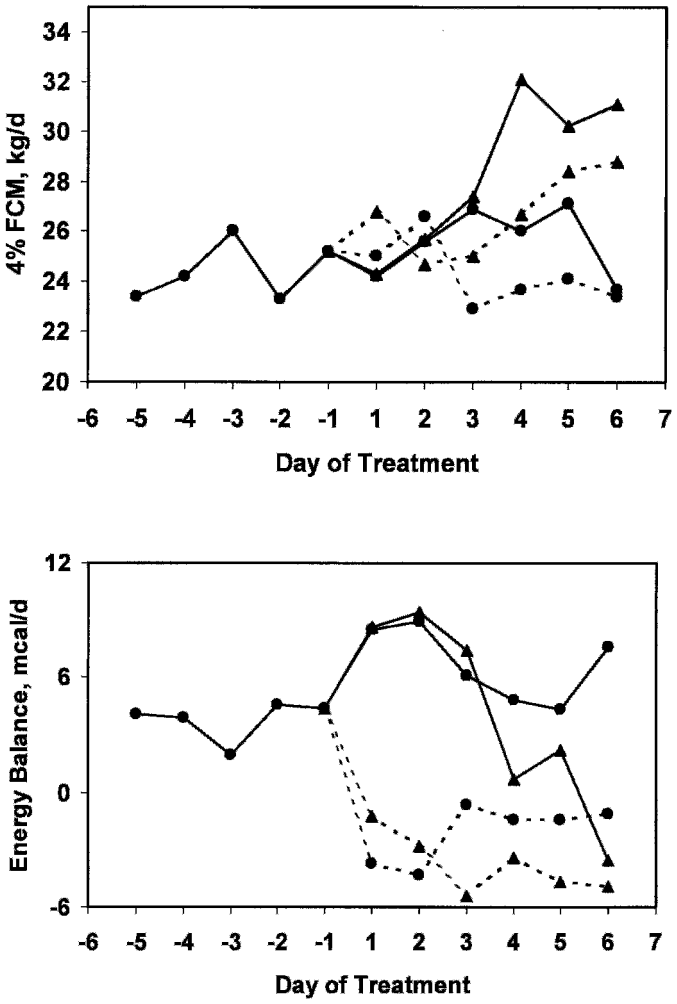

Figure 1. Effect of bST and feed restriction on milk production and tissue energy balance. Cows were fed ad libitum or intake was restricted to 80% of ad libitum consumption. Hormone and control periods were 6 d with 15 d recovery between treatment periods. Triangles = bST, circles = no hormone, solid line = ad libitum feeding, dashed line = restricted feeding.

Our data suggest that the experimental paradigm used in this study did not mimic the negative energy balance of a high production dairy cow during early lactation. The negative energy balance observed in early lactation is one in which energy intake lags behind energy needs for milk production, whereas in this experiment, feed energy was restricted and milk energy output was maintained during the 6-d period. Furthermore, the hypothyroid state of lactating animals, particularly during peak lactation and during stimulation with bST, resembles that of a syndrome described in humans that is known as "low T3 syndrome" or "euthyroid sick syndrome" (Aceves et al., 1985). The syndrome is characterized by altered peripheral deiodinase patterns and normal thyroid function, resulting in a low plasma concentration of T_3 and an increased concentration of reverse T_3. The syndrome has been noted in a number of systemic nonthyroidal catabolic illnesses and during acute caloric deprivation (Chopra et al., 1978; Wartofsky and Burman, 1982). Hormonal adjustments characteristic of the syndrome apparently serve to reduce energy expenditure in the organism and TNF has been implicated in the etiology of the syndrome. TNF inhibits 5′D and the synthesis and secretion of T_3 and T_4 in humans and rodents (Ozawa et al., 1988; Sato et al., 1990; Tang et al., 1995; van der Poll et al., 1990). In the present study, the concentration of TNF in the serum was not affected by feed restriction or by bST. Although an increase in plasma TNF concentration did not accompany the reduction in circulating concentrations of thyroid hormones, TNF may inhibit 5′D and thyroid hormone secretion in dairy cows because many basal metabolic effects of cytokines such as TNF are often induced in a paracrine manner by resident monocytes. Furthermore, bST did not elevate plasma TNF, which suggests that the hormone does not produce a systemic stress response.

The influence of bST treatment on production of cytokines has not been reported previously, although bST has been reported to promote recovery from bovine mastitis. There is no adverse effect of bST on the health of treated cows (Burton et al., 1994). Small increases in milk somatic cell count may occur in response to bST administration even in the absence of mastitis (McBride et al., 1990) and may be the result of altered neutrophil trafficking. Vandeputte-Van Messom and Burvenich (1993) demonstrated that cows that had been experimentally infected with coliform mastitis or *Streptococcus uberis* recovered mammary function more quickly when treated with bST (Hoeben et al., 1999). Furthermore, bST treatment appeared to reduce the incidence of mastitis spreading to previously nonin-

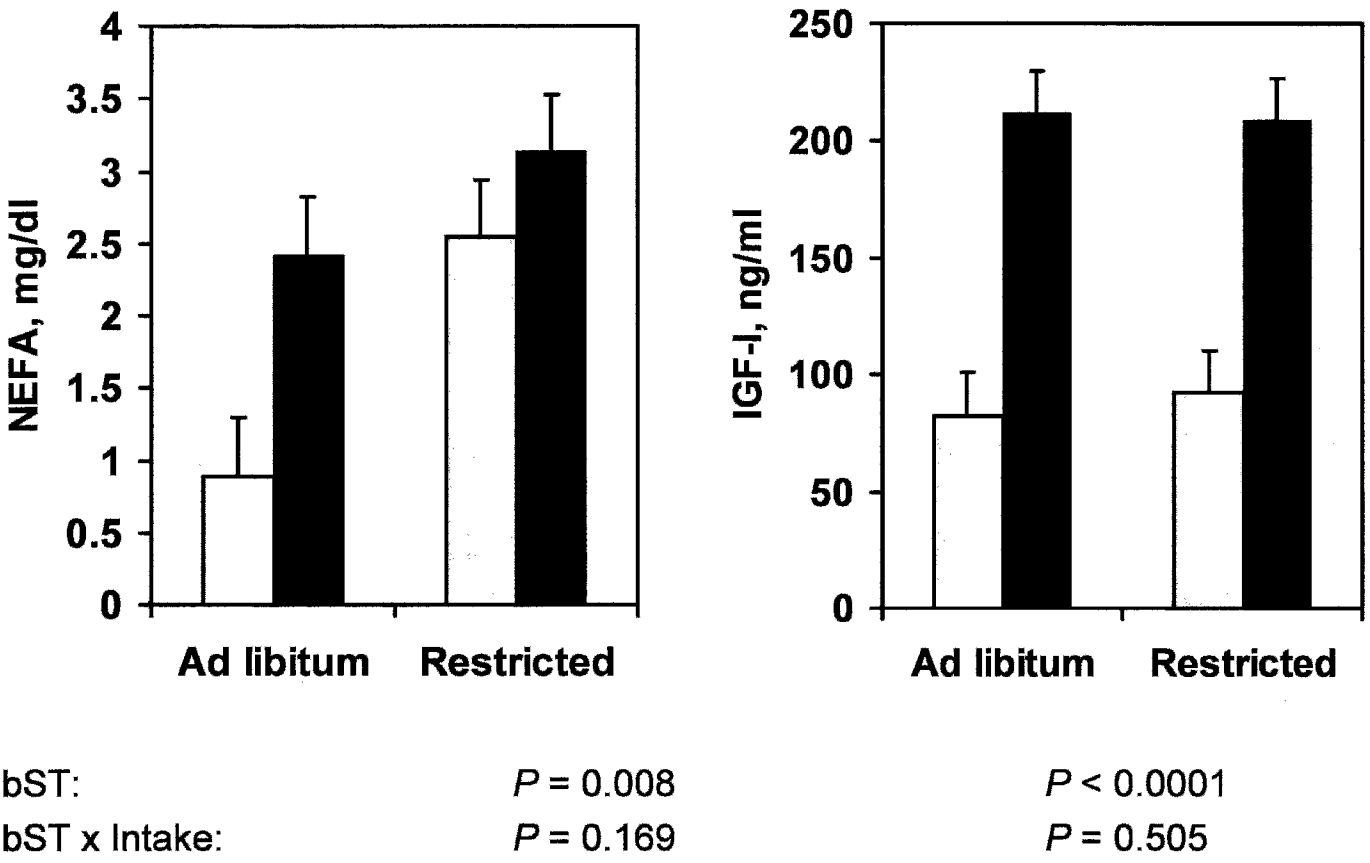

Figure 2. Effect of bST and feed restriction on plasma NEFA and IGF-I. Cows were fed ad libitum or intake was restricted to 80% of ad libitum consumption. Hormone and control periods were 6 d with 15-d recovery between treatment periods. NEFA concentration was determined on serum obtained on d 6 of control and bST treatment periods. IGF-I concentrations are means for a morning and evening serum sample on d 6 of control and bST treatment periods. Bars indicate means ± SE for six cows. Open bars = control period, solid bars = bST treatment period.

fected quarters. The present report provides evidence that these effects are not mediated by increases in serum TNF.

During lactation of rats, 5′D decreases in liver and kidney, and increases in mammary gland (Kahl et al., 1987; Valverde-R and Aceves, 1989). These organ-specific changes are proportional to lactation intensity (Jack et al., 1994; Valverde-R and Aceves, 1989) and are probably an important aspect of physiological adjustments necessary to support lactation. They are consistent with focusing metabolic priority on lactating mammary glands by maintaining them in a euthyroid state, despite a systemic decrease in thyroid hormone concentrations. Analogous organ-specific changes in 5′D occur with onset of lactation in ruminants (Capuco et al., unpublished data).

Different enzymes with 5′-deiodinase activity exist, and their characteristics have been summarized (Leonard and Visser, 1986). The 5′D present in bovine mammary tissue is a type II 5′D, whereas that expressed in liver is a type I 5′D (Kahl et al., 1993). Although the 5′D in rat mammary gland is a type I 5′D (Aceves and Valverde, 1989), the gene is distinct from that expressed in liver (Jack et al., 1994; Navarro et al., 1997). Indeed, in all species examined, the mRNA for mammary 5′D appears to differ from that in liver. This is consistent with the unique regulation of mammary 5′D during lactation. We have recently demonstrated that mammary 5′D activity in thyroid-ablated, lactating mice was increased by administration of either bovine prolactin or bST, whereas hepatic 5′D was unaffected (Capuco et al., 1999). Similarly, the diminished mammary 5′D activity 12 h after cessation of suckling was restored by administration of prolactin or norepinephrine (Aceves et al., 1999). Thus, the galactopoietic hormones and the neuroendocrine response to suckling may provide signals for increasing 5′D in mammary tissue during lactation in rodents. Additional study is necessary to determine if the same holds true for ruminants.

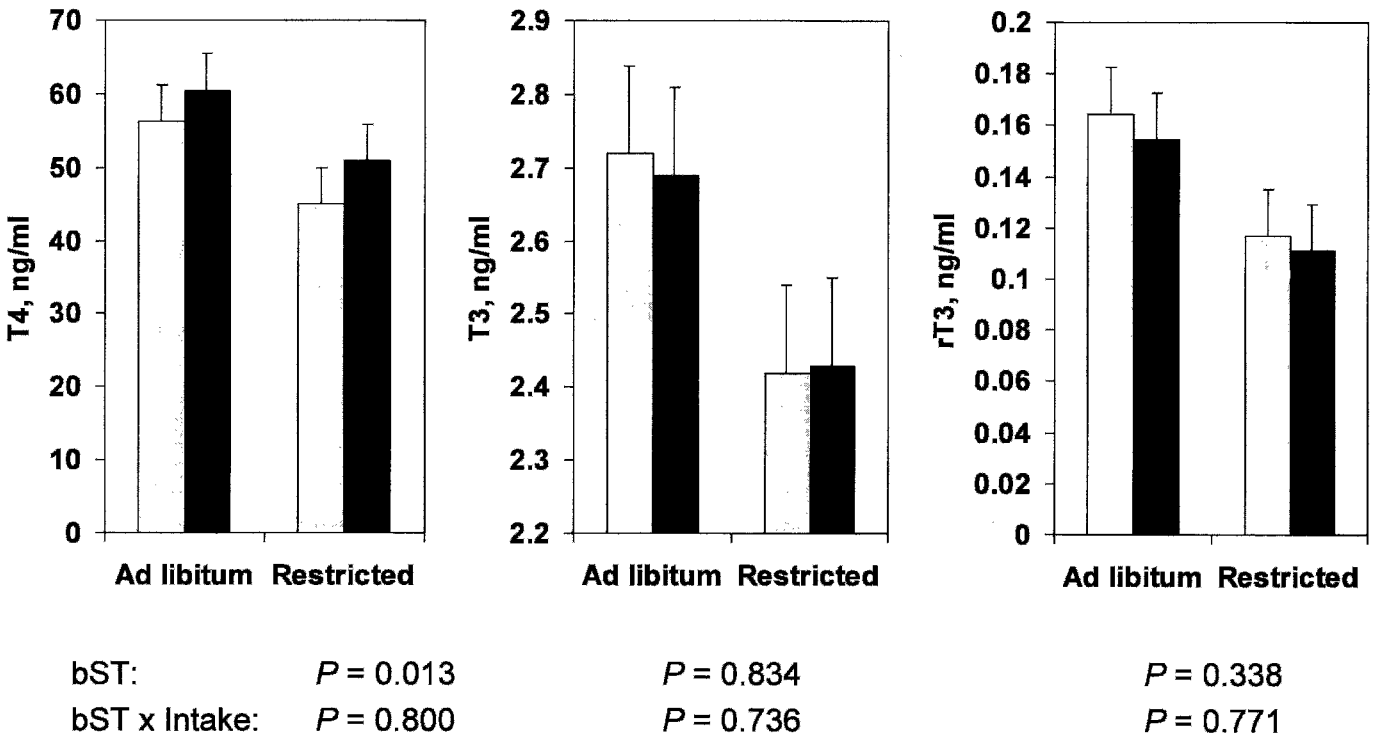

Figure 3. Effect of bST and feed restriction on serum concentrations of thyroxine (T_4), triiodothyronine (T_3) and reverse-triiodothyronine (rT_3). Cows were fed ad libitum or intake was restricted to 80% of ad libitum consumption. Hormone and control periods were 6 d with 15-d recovery between treatment periods. Hormone concentrations are mean values for serum samples obtained every 2 h for 12 h on day 6 of control and bST treatment periods. Bars indicate means ± SE for six cows. Open bars = control period, solid bars = bST treatment period.

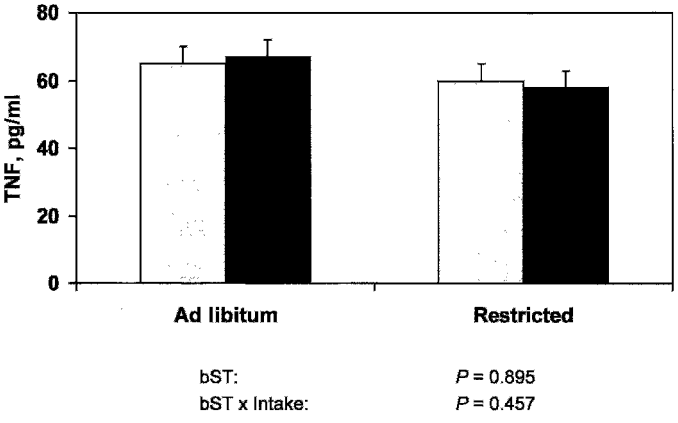

Figure 4. Effect of bST and feed restriction on serum tumor necrosis factor-α (TNF). Cows were fed ad libitum or intake was restricted to 80% of ad libitum consumption. Hormone and control periods were 6 d with 15 d recovery between treatment periods. TNF concentration was determined on four serum samples collected during a 12-h sampling on day 6 of control and bST treatment periods. Bars indicate means ± SE for 6 cows, open bars = control period, solid bars = bST treatment period.

Previous data had indicated that administration of bST to dairy cows resulted in organ-specific changes in 5′D that were consistent with enhancing metabolic priority of the mammary gland. These data also suggested an influence of lactation stage or energy balance. When bST was administered (40 mg/d, 5 d) to cows late (270 d) in first lactation, 5′D in mammary tissue nearly doubled, but activity was not significantly altered in liver or kidney (Capuco et al., 1989). In a subsequent experiment (Kahl et al., 1995), cows were continuously infused intravenously (12 mg/d, 63 d) with bST or growth hormone-releasing factor beginning on day 120 of lactation. Treatment with somatotropic hormones resulted in decreased hepatic 5′D and enhancement of the hypothyroid state (reduced serum T_3 and decreased T_3/T_4 ratio). However, activity of mammary 5′ D was not affected. Thus, we proposed that bST enhances thyroid hormone activity within the mammary gland, relative to that in other peripheral tissues. This may be accomplished by an increase in 5′D in mammary gland or a decrease in 5′D in liver, with the nature of the response dependent upon energy balance.

The present study attempted to evaluate energy balance as a variable that impacts the nature of the 5′D

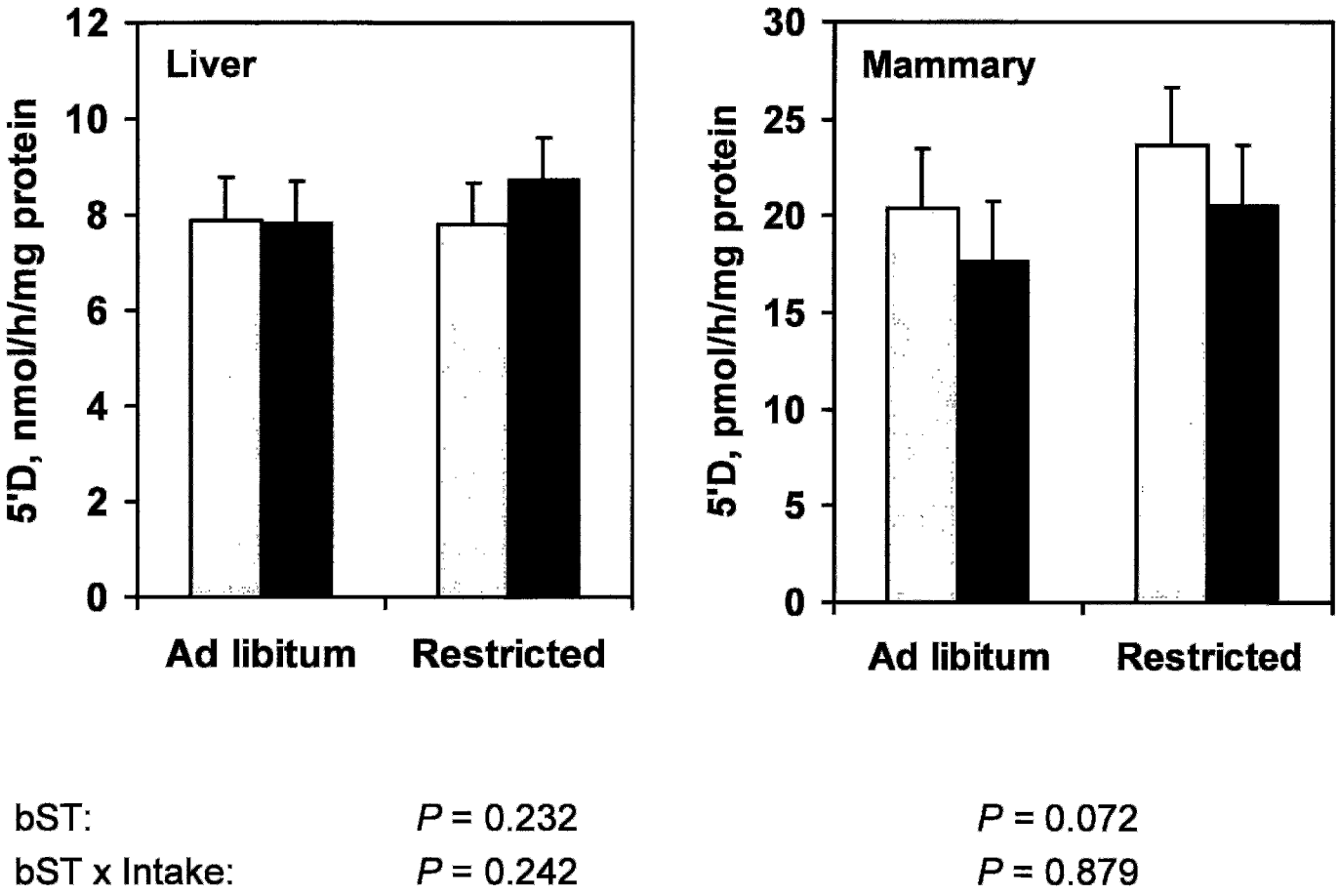

Figure 5. Effect of bST and feed restriction on liver and mammary thyroxine 5′-deiodinase (5′D) activity. Cows were fed ad libitum or intake was restricted to 80% of ad libitum consumption. Hormone and control periods were 6 d with 15-d recovery between treatment periods. Tissue was obtained on day 6 of treatment and control periods by mammary biopsy, ≈23 h after injection of hormone or vehicle. Bars indicate means ± SE for six cows. Open bars = control period, solid bars = bST treatment period.

response to bST administration. However, no alteration in 5′D was elicited by bST, regardless of energy balance. This may have been due to differences in sampling or treatment protocols between this and previous experiments. Recent investigations indicate that somatotropin and prolactin increase 5′D and are galactopoietic in mice only when thyroid hormones are present (Capuco et al., 1999). The relationship between bST and thyroid hormone metabolism requires additional study. It is clear that alterations in 5′D activity in peripheral tissues plays an important role in the physiological adjustment to support lactation in rodents. Alterations in 5′D activity likely plays a similar role for initiation of lactation in dairy cows but may not be as important in regulating the intensity of an established lactation.

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