# Slow Release Somatotropin in Dairy Heifers and Cows Fed Two Levels of Energy Concentrate. 2. Plasma Hormones and Metabolites

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## ABSTRACT

Fifty Holstein dairy cows (26 primiparous) were used to evaluate effects of level of concentrate and of slow release recombinant bST on plasma hormones and metabolites. Blood was sampled at wk 14 and 20 of lactation, which was 5 and 11 wk after the first bST injection. In addition, at d 3 and 10 after the third bST injection, diurnal profiles of somatotropin and metabolites were studied in 7 bST cows and 5 control cows by blood sampling every 20 min over 6 h. Supplementation by bST enhanced plasma somatotropin and insulin-like growth factor-I but did not significantly affect plasma concentrations of triiodothyronin and insulin. The bST supplementation increased plasma NEFA at wk 14 and reduced uremia at wk 20. Primiparous cows showed higher plasma NEFA and triiodothyronin than multiparous cows at both sampling periods, higher insulin-like growth factor I, and lower β-hydroxybutyrate at wk 14, and higher glucose and lower insulin at wk 20. In the diurnal kinetic study, bST supplementation did not alter bST spike frequency and duration but increased spike magnitude, the area under the curve above the baseline, and the baseline mean. Sixty-one to 56% of the increase over controls in plasma bST total area was due to increase in the area under the curve above baseline. Preprandial NEFA were increased by bST at d 10.

(Key words: dairy cows, somatotropin, hormones, metabolites)

**Abbreviation key:** BHBA = β-hydroxybutyrate, IGF-I = insulin-like growth factor-I, RIA = radioimmunoassay, $T_3$ = triiodothyronin.

## INTRODUCTION

Administration of exogenous bST increases milk yield in dairy cows up to 40% with large interassay variations (15). Often, but not always, interactions of bST effects with nutritional factors and parity of the cows (6, 7) occur. Although the mechanism by which bST stimulates milk yield is not completely understood, it is assumed that stimulation of mammary metabolism is mediated in part by increasing hepatic production of insulin-like growth factors, especially factor-I (IGF-I) (15, 19). Nutritional factors were shown to modify hepatic bST receptors in steers (5). Furthermore, plasma IGF-I concentrations were related positively to nutritional status in steers (5) and to energy balance or lactation stage in lactating cows (21, 24). On the other hand, bST is known to alter extramammary metabolism to provide the necessary nutrients to the mammary gland (15). Additional nutrients also result from increased feed intake that generally occurs several weeks after the start of bST supplementation.

Endogenous bST secretion was shown to be pulsatile in steers (11) and lactating cows (22, 31). However, there are very few published data related to bST patterns on a day to day and hour to hour basis after injections of slow release bST formulations (24, 27, 32). The aims of the present study were to investigate the effects of slow release bST (injected every 14 d) on several plasma hormones and metabolites in primiparous and multiparous cows receiving

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two levels of concentrate (20), depending on the duration of bST supplementation (5 vs. 11 wk after the first injection), and to examine the effects of days elapsed after bST injection (3 vs. 10 d) and of time after feeding on bST pattern and metabolite profiles assessed by frequent blood sampling over 6 h.

## MATERIALS AND METHODS

### Animals and Experimental Design

Fifty Holstein dairy cows (26 primiparous) received corn silage ad libitum and high or low level of concentrate from wk 3 of lactation until the end of the winter period [see Rémond et al. (20) for more details]. From wk 9 postpartum, half of each nutritional group was submitted every 14 d to a subcutaneous injection of 500 mg of recombinant methionyl bST in a prolonged release preparation (Sometribove, Monsanto, St. Louis, MO) or placebo for controls.

At wk 2, 6, 14 (SD = 1), i.e., 9 ± 3 d after the third injection, and 20 (SD = 1), i.e., 11 ± 5 d after the sixth injection, (postpartum), blood was sampled from each cow before morning feeding via tail venipuncture into two 5-ml vacutainers (Venoject; Terumo, 3030 Leuven, Belgium). One vacutainer contained 25 units of lithium heparinate, and the other contained 7 mg EDTA. Blood was centrifuged immediately at 3000 × *g* for 20 min. Heparinized plasma (for metabolite measurements) and EDTA-plasma (for hormone measurements) were divided into .6-ml aliquots. Thirty microliters of iniprol ($10^6$ kallicrein inhibitor units/5 ml; Choay, 75782 Paris, France) also were added to EDTA-plasma samples intended for bST and IGF-I determinations. Plasma samples were stored at –20°C until subsequent analysis.

Moreover, diurnal profiles were studied in 7 bST-treated cows (2 multiparous) and 5 primiparous control cows from the high concentrate group at 3 and 10 d after the third injection of bST or placebo. Due to agitation, one cow (number 84006) did not receive the complete dose of bST. Animals were milked between 0600 and 0800 h. A catheter was inserted into the right external jugular vein and filled with heparinized isotonic saline until sampling began the next day. Blood was taken from 0820 to 1420 h in EDTA-coated tubes. Sampling interval was 20 min in order to detect spikes of bST endogenous secretion. Exact time of feed distribution (between 0900 and 1030 h) was recorded for each cow.

### Hormone and Metabolite Assays

Plasma immunoreactive somatotropin was analyzed by radioimmunoassay (RIA) (10). No significant crossreaction was found with highly purified bovine prolactin (NIH $PB_2$) or purified bovine chorionic somatomammotropin. Sensitivity was .4 ng/ml and variations within and between assays were 5.5 and 6.2%, respectively (10). Plasma IGF-I was measured by RIA after extractions. In order to dissociate and separate IGF-I from its carrier protein, plasma was mixed with four volumes .5 *M* HCl and incubated in glass tubes for 1 h at room temperature. After incubation, an octadecylsilane-silica extraction was performed (Sep Pack C18 cartridge; Waters Associates, Milford, MA). Recovery of pure recombinant human IGF-I (Ciba-Geigy, Basel, Switzerland) was 91%. Concentrations less than 15 ng/ml could not be detected. Variations within and between assays were respectively 8 and 12% (10). Triiodothyronin ($T_3$) was measured by RIA using Amerlex-M $T_3$ RIA kit (Amersham, Les Ulis, France). Variations within and between assays were 1.6 and 3.2%, respectively. The sensitivity of the assay was .15 nmol/L. Plasma insulin was determined using a INSIK-1M RIA kit (ORIS Industry, Gif-sur-Yvette, France). Assay sensitivity was 3 μU/ml, and variations within and between assays were 9 and 13%, respectively.

Plasma glucose was assayed by the glucose dehydrogenase method (Merckotest kit, reference number 3389; Merck, Nogent-sur-Marne, France). Nonesterified fatty acids were assayed automatically (8), using an enzymatic Wako kit (Biolyon, 69572, Dardilly, France). β-Hydroxybutyrate (BHBA) was determined automatically (1). Urea was measured by the urease method (kit reference number 489620; Boehringer-Mannheim, Meylan, France).

### Statistical Methods

Data from wk 14 and 20 postpartum were examined by ANOVA using the following linear model:

$$Y_{ijke} = \mu + A_i + B_j + C_k + AB_{ij} + AC_{ik} + BC_{jk} + e_{ijke}$$

where $Y_{ijke}$ = dependent variable; $\mu$ = overall mean; $A_i$ = effect of bST supplementation (bST or placebo, 1 df); $B_j$ = effect of level of concentrate (high or low, 1 df); $C_k$ = effect of parity (multiparous or primiparous, 1 df); $AB_{ij}$ = bST × concentrate level interaction (1 df); $AC_{ik}$ = bST × parity interaction (1 df); $BC_{jk}$ = concentrate level × parity interaction (1 df); and $e_{ijke}$ = residual (43 df). Logarithmic transformation was applied on some traits (Table 1) to equalize variances for statistical analysis. For each variable, within-group differences between wk 14 and wk 20 also were assessed using the paired *t* test. Data from wk 2 and 6 of lactation were used only for the analysis of plasma IGF-I variation (Figure 1).

The bST pattern over a 6-h period was analyzed using the Pulsar program (14), which allows estimations of the number, duration, and maximal magnitude of spikes and the baseline between spikes. Values for peak cutoff criteria (G values) were G1, 2.3; G2, 1.3; G3 to G5, 1.0. Differences between groups (bST versus control) or within-group differences between days (3 vs. 10) were assessed respectively by Student's *t* test and by paired *t* test. Effects on plasma metabolites of bST treatment, day of sampling after bST injection, and time after feeding (15 measures from −50 to 230 min) were tested by ANOVA using the following model [repeated measures (23)]: treatment (effect 1, bST versus control, 1 df), cow within treatment (error 1, 10 df), day (effect 2, 1 df) day × treatment (effect 3, 1 df), day × cow within treatment (error 2, 10 df), time (effect 4, 14 df), time × treatment (effect 5, 14 df), time × cow within treatment (error 3, 140 df), day × time (effect 6, 14 df), day × time × treatment (effect 7, 14 df), and day × time × cow within treatment (error 4, 140 df). Effect 1 was tested on error 1; effects 2 and 3 were tested on error 2; effects 4 and 5 were tested on error 3; and effects 6 and 7 were tested on error 4. Probabilities of 10, 5, and 1% were used.

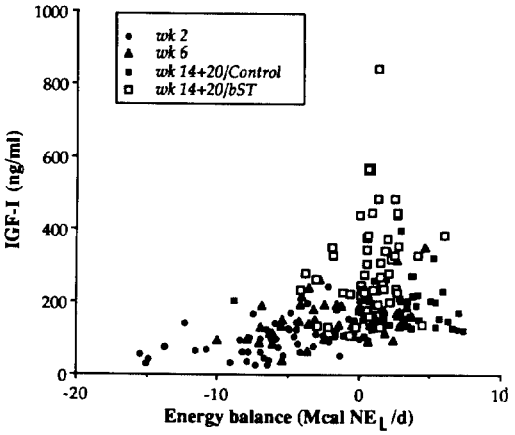


Figure 1. Relationships between plasma insulin-like growth factor I (IGF-I) and energy balance at wk 2, 6, 14, and 20 of lactation (50 cows/wk); bST supplementation started at wk 9 (26 bST-supplemented cows and 24 controls). Linear adjustment:

$y = 195 + 10.9x$; $r = .45$ in all cows ($n = 200$).
$y = 158 + 7.5x$; $r = .55$ in controls ($n = 148$).
$y = 281 + 15.2x$; $r = .23$ in bST-supplemented cows ($n = 52$).

Exponential adjustment:

$y = 169 \times 10^{(.034x)}$; $r = .59$ in all cows.
$y = 144 \times 10^{(.028x)}$; $r = .61$ in controls.
$y = 257 \times 10^{(.023x)}$; $r = .25$ in bST-supplemented cows.

## RESULTS

### Changes in Preprandial Plasma Concentrations of Hormones and Metabolites

*Hormones.* As shown in Table 1, the bST concentration in plasma was not significantly affected at wk 14 or 20 by parity or level of concentrate. Circulating bST was about sevenfold higher in cows receiving sometribove than in controls at both weeks. Concentrations were higher, although not significantly so, at wk 20 versus 14 of lactation.

The IGF-I concentration in plasma was not significantly affected by concentrate level; bST supplementation caused a significant twofold increase in circulating IGF-I concentration at both weeks with a great variability of individual values. Plasma IGF-I at wk 14 was higher in primiparous than in multiparous cows. At wk 20, bST effect on IGF-I concentration was greater in multiparous than in primiparous cows (significant interaction). When all data were pooled (wk 2, 6, 14, and 20 of lactation), plasma IGF-I concentration was related positively to the energy balance [calculated by Rémond et al. (20)] with a better relationship for control than for bST cows (Figure 1). Exponen-

TABLE 1. Plasma hormones and metabolites during bST or placebo administration.

| | Multiparous[1] | | | | Primiparous[1] | | | | Estimated effects[2,3] | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | High concentrate | | Low concentrate | | High concentrate | | Low concentrate | | | | | | | | |
| | bST | Control | bST | Control | bST | Control | bST | Control | P | | C | | bST | | Residual[4] |
| Number of cows | 6 | 5 | 6 | 7 | 7 | 6 | 7 | 6 | | | | | | | |
| wk 14 (SD = 1) of lactation | | | | | | | | | | | | | | | |
| Milk yield, kg/d | 28.0 | 26.8 | 29.2 | 24.5 | 23.2 | 19.5 | 23.3 | 21.9 | 5.1 | ** | .3 | NS | 2.8 | * | 4.7 |
| Energy balance,[5] Mcal/d | −.8 | 1.3 | −.1 | 2.1 | 1.6 | 4.7 | .7 | −.5 | −.9 | NS | 1.1 | NS | −1.6 | * | 2.6 |
| Protein balance, g/d | −91 | −10 | −11 | 83 | −18 | 133 | −26 | −29 | −20 | NS | 2 | NS | −83 | † | 180 |
| bST, ng/ml | 15 | 3 | 14 | 2 | 20 | 3 | 20 | 2 | .84 | NS | 1.2 | NS | 6.8 | ** | 37 |
| IGF-I, ng/ml | 222 | 151 | 262 | 139 | 312 | 200 | 284 | 191 | .79 | * | 1.0 | NS | 1.5 | ** | 12 |
| $T_3$, nmol/ml | 1.25 | 1.33 | 1.51 | 1.43 | 1.76 | 1.63 | 1.53 | 1.66 | .83 | ** | .97 | NS | 1.0 | NS | 5 |
| Insulin, μU/ml | 20 | 18 | 20 | 17 | 21 | 22 | 18 | 23 | −2.1 | NS | .7 | NS | −.2 | NS | 5 |
| Glucose, mg/dl | 66 | 66 | 68 | 69 | 71 | 68 | 64 | 69 | −.7 | NS | .5 | NS | −.9 | NS | 6 |
| NEFA, m*M* | .23 | .10 | .18 | .13 | .35 | .19 | .51 | .15 | .60 | ** | 1.0 | NS | 1.9 | ** | 45 |
| BHBA, m*M* | .57 | .44 | .71 | .57 | .44 | .44 | .50 | .49 | 1.2 | ** | .82 | ** | 1.1 | NS | 6 |
| Urea, mg/L | 243 | 305 | 298 | 271 | 222 | 247 | 229 | 286 | 32 | NS | −16 | NS | −28 | NS | 61 |
| wk 20 (SD = 1) of lactation | | | | | | | | | | | | | | | |
| Milk yield, kg/d | 24.3 | 23.6 | 26.0 | 22.8 | 22.0 | 17.6 | 21.7 | 20.0 | 3.8 | ** | −.7 | NS | 2.6 | * | 4.4 |
| Energy balance, Mcal/d | 1.4 | 3.2 | 1.0 | 3.9 | 2.1 | 4.3 | .1 | 2.8 | .2 | NS | 1.1 | † | −2.2 | ** | 1.9 |
| Protein balance, g/d | −72 | 22 | −60 | 13 | −46 | 92 | −115 | 60 | −22 | NS | 25 | NS | −120 | ** | 107 |
| bST, ng/ml | 27 | 6 | 26 | 6 | 29 | 4 | 28 | 4 | 1.1 | NS | 1.2 | NS | 6.3 | ** | 18 |
| IGF-I,[6] ng/ml | 337 | 136 | 390 | 163 | 269 | 247 | 276 | 174 | 1.1 | NS | 1.0 | NS | 1.7 | ** | 17 |
| $T_3$,[5] nmol/ml | 1.62 | 1.44 | 1.88 | 1.70 | 2.29 | 2.10 | 1.80 | 1.90 | .93 | ** | 1.0 | NS | 1.1 | NS | 3 |
| Insulin, μU/ml | 26 | 21 | 23 | 20 | 19 | 22 | 22 | 17 | 3.0 | † | 1.6 | NS | 2.8 | NS | 6 |
| Glucose, mg/dl | 68 | 68 | 62 | 64 | 71 | 69 | 68 | 64 | −2.3 | † | 4.4 | ** | .7 | NS | 4 |
| NEFA, m*M* | .09 | .08 | .10 | .07 | .13 | .10 | .15 | .11 | .64 | ** | .93 | NS | 1.2 | NS | 26 |
| BHBA, m*M* | .55 | .45 | .73 | .57 | .50 | .46 | .52 | .63 | 1.1 | NS | .79 | ** | 1.1 | NS | 10 |
| Urea, mg/L | 199 | 279 | 212 | 229 | 185 | 241 | 168 | 205 | 29 | NS | 23 | NS | −47 | * | 48 |

[1]Unadjusted means. Weeks 14 and 20 = 9 ± 3 and 11 ± 5 d after the third and sixth bST or placebo injections, respectively.

[2]A logarithmic transformation was employed for statistical analysis of plasma NEFA, β-hydroxybutyrate (BHBA), bST, insulin-like growth factor-I (IGF-I), and triiodothyronin ($T_3$).

[3]P = Parity (multiparous versus primiparous), C = level of concentrate (high versus low) and bST (bST versus control).

†$P < .10$, *$P < .05$, **$P < .01$, or NS ($P > .10$). Effects are differences for nontransformed variables and ratios of antilog values coming from means of log-transformed variables.

[4]Standard deviation for nontransformed variables or coefficient of variation in percentage for log-transformed variables.

[5]Parity × level of concentrate interaction significant for energy balance ($P < .05$) and for plasma $T_3$ ($P < .01$).

[6]bST × parity interaction significant ($P < .05$).

tial adjustment was better than linear adjustment.

The $T_3$ concentrations were not modified by level of concentrate or bST supplementation. Primiparous cows had higher concentrations of plasma $T_3$ at both weeks, and at wk 20 the higher level of concentrate increased plasma $T_3$ in primiparous cows and decreased it in multiparous cows. Insulin concentration was not significantly affected by concentrate or bST supplementation. Multiparous cows had slightly higher concentrations of insulin than primiparous cows at wk 20.

*Metabolites.* Glucose concentration in plasma was not significantly affected by administration of bST at either week. Glycemia did not significantly differ among groups at wk 14 but was higher at wk 20 in the high versus low concentrate groups and in primiparous rather than in multiparous cows. Concentration of NEFA in plasma was not modified by concentrate level at either wk 14 or 20. Concentrations of plasma NEFA were twofold increased in bST-supplemented cows at wk 14, and this effect persisted, but not significantly, at wk 20. Primiparous cows had higher concentrations of plasma NEFA than multiparous cows at both weeks.

Concentration of BHBA in plasma was not significantly affected by bST at wk 14 or 20 of lactation, but it was higher in the low versus high concentrate group at both weeks. This trait also was higher in multiparous than in primiparous cows at wk 14. Plasma BHBA was negatively correlated with glycemia at wk 20 ($r = -.55$, $n = 50$). Uremia was not affected by concentrate level; bST supplementation caused a decline in plasma urea that was significant at wk 20. At this week, uremia was correlated positively ($r = .46$, $n = 50$) with protein balance [expressed in a new protein feeding system: protein digestible in the intestine, calculated as by Rémond et al. (20)].

### Diurnal Plasma Profiles of Somatotropin and Metabolites

*Somatotropin.* The bST pattern in control cows was episodic at both 3 and 10 d after third placebo injection (Figure 2). There were wide differences between individuals in spike magnitude (from .7 to 6.5 ng/ml at d 3 and from .8 to 15.9 ng/ml at d 10), numbers (3 to 6), and duration (20 to 120 min). Administration of exogenous bST did not alter either spike frequency or total duration at both days (Table 2) and did not suppress asynchronism in spike incidence (Figures 2 and 3). However, bST markedly increased spike magnitude (from 4.1 to 45.2 ng/ml at d 3 and from 3.2 to 83.6 ng/ml at d 10), the area under the curve, and the baseline mean (Table 2). Values were much greater at d 10 than at d 3.

The increase in area under curve above the baseline and the increase in the area under the

TABLE 2. Plasma somatotropin kinetic traits at d 3 and 10 after the third injection of bST or placebo.[1,2]

| | Day 3 | | | | Day 10 | | | |
|---|---|---|---|---|---|---|---|---|
| | Control | | bST | | Control | | bST | |
| Cows, n | 5 | | 7 | | 5 | | 7 | |
| | $\bar{X}$ | SD | $\bar{X}$ | SD | $\bar{X}$ | SD | $\bar{X}$ | SD |
| Overall mean, ng/ml | 1.8 | 1.0 | 12.4** | 6.0 | 1.9 | 1.4 | 20.0**,[b] | 8.7 |
| Spike frequency over 6 h | 3.8 | .4 | 4.7 | 1.2 | 4.2 | .8 | 4.4 | .9 |
| Total spike duration, min | 188 | 75 | 243 | 76 | 228 | 27 | 234 | 30 |
| Spike maximal magnitude, ng/ml | 2.6 | 1.4 | 17.8** | 8.4 | 2.9 | 2.7 | 33.2**,[a] | 13.2 |
| Baseline mean, ng/ml | 1.3 | .5 | 7.7* | 5.7 | 1.2 | .5 | 10.4** | 5.4 |
| Total area under curve,[3] ng/ml per h | 108 | 61 | 744** | 351 | 122 | 89 | 1151**,[b] | 520 |
| Area under curve above baseline,[3] ng/ml per h | 30 | 35 | 282** | 124 | 50 | 59 | 527**,[b] | 216 |

[1]Values of bST group different from control: *$P < .05$ or **$P < .01$.

[2]Values at d 10 differ from d 3: [a]$P < .01$, [b]$P < .05$ (paired $t$ test).

[3]Integrated area of plot of concentrations over a 6-h period.

baseline represented, respectively, 61 and 39% of the total area increase in bST versus control cows at d 3 and 56 and 44% at d 10 (Table 2 and Figures 2 and 3). From d 3 to d 10, the total area under the curve in bST-supplemented cows increased up to 60%. About half (45%) of this increase was due to increase in the area under curve above the baseline and half (55%) to increase in the area under the baseline.

*Metabolites.* Morning feeding was followed by decreases in plasma glucose and NEFA concentrations and increases in BHBA and urea (Figure 4 and Table 3). Glucose and BHBA concentrations were not significantly affected

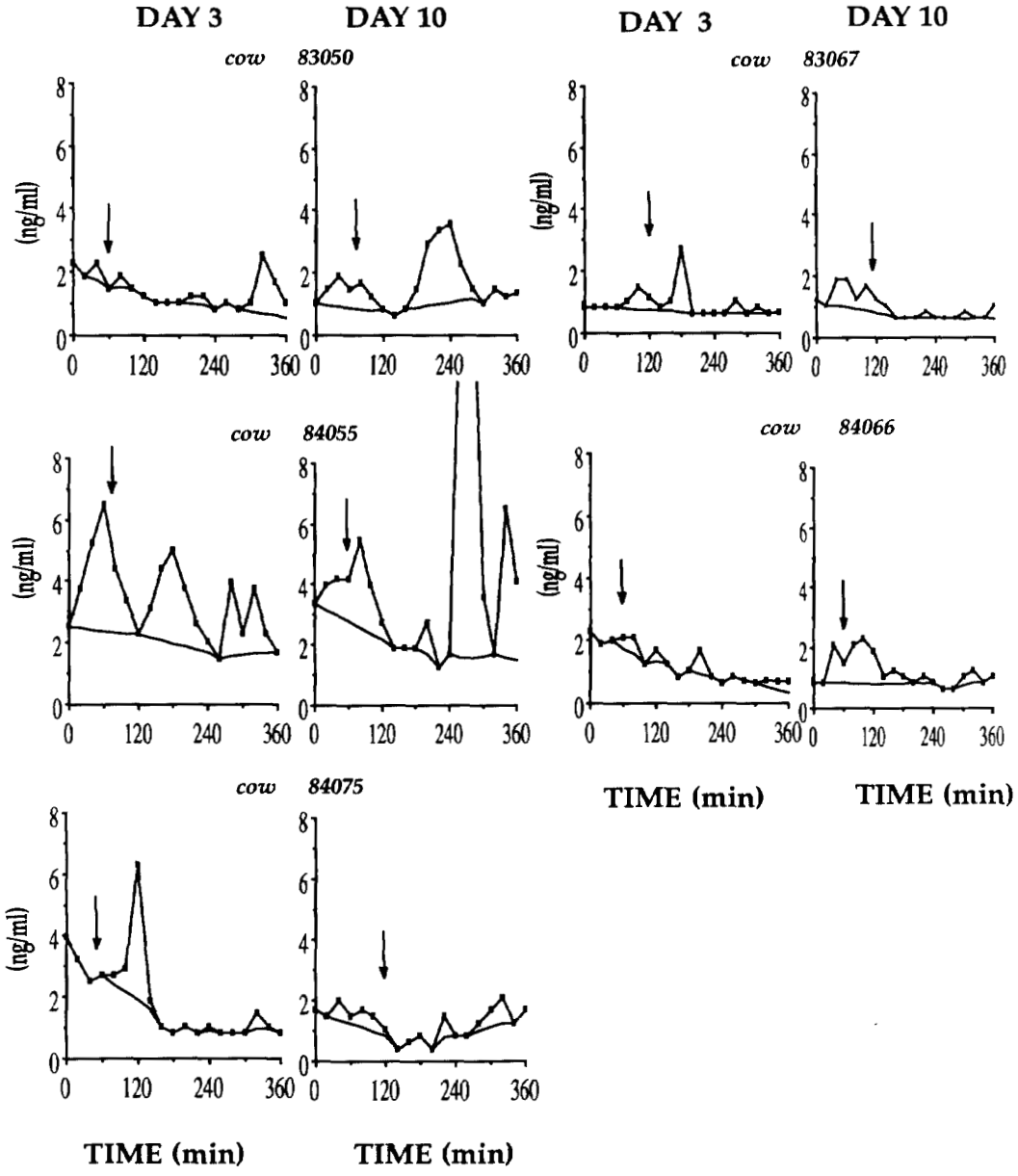


Figure 2. Individual plasma bST profiles of 5 control cows at d 3 and 10 after the third injection of placebo. (■ = concentration; — = baseline; arrows indicate time of feeding.)

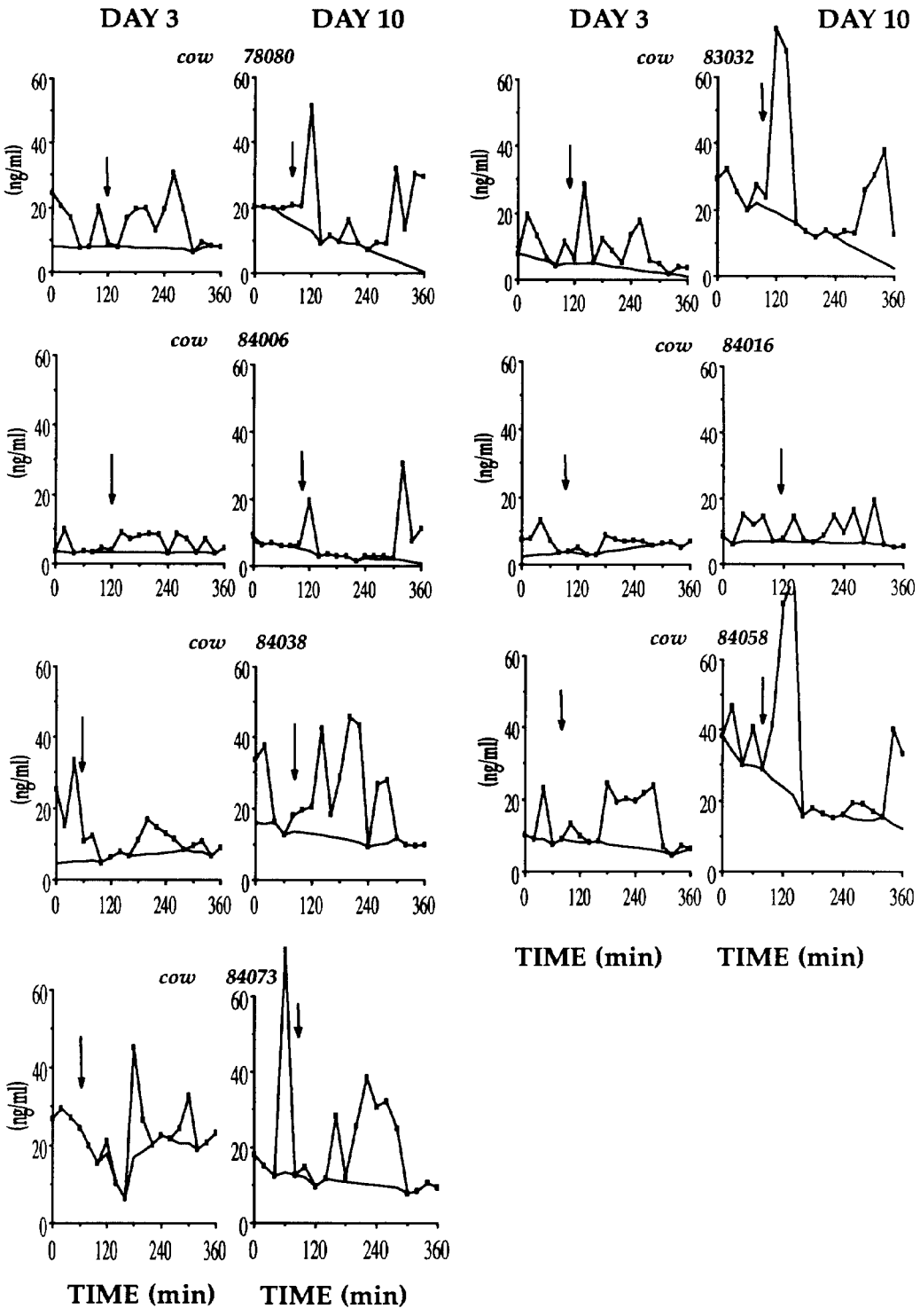


Figure 3. Individual plasma bST profiles of 7 bST-supplemented cows at d 3 and 10 after the third injection of sometribove. (■ = concentration; — = baseline; arrows indicate time of feeding.)

TABLE 3. Plasma metabolites at d 3 and 10 after the third injection of bST or placebo.

| | Day 3[1] | | Day 10[1] | | Estimated effects[2] | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Control | bST | Control | bST | bST[3] | | Day[4] | | Time[5] | | bST × day[6] | bST × time[7] | Residual SD |
| Cows, n | 5 | 7 | 5 | 7 | | | | | | | | | |
| Glucose, mg/dl | 61.6 | 62.7 | 62.9 | 62.5 | .4 | NS | .6 | NS | −2.0 | ** | NS | NS | 4.0 |
| NEFA, $mM$[8] | .15 | .15 | .13 | .22 | .05 | † | .03 | NS | −.05 | ** | † | * | .09 |
| BHBA, $mM$ | .51 | .54 | .52 | .56 | .04 | NS | .01 | NS | .13 | ** | NS | NS | .16 |
| Urea, mg/L[9] | 290 | 262 | 323 | 274 | −38 | NS | 23 | NS | 19 | ** | NS | NS | 46 |

[1]Nonadjusted means (from 50 min before to 230 min after feeding).

[2]†$P < .10$, *$P < .05$, **$P < .01$.

[3]bST less control.

[4]D 10 less d 3.

[5]Slope (units per hour after feeding) of the linear regression, after removal of factor effects.

[6]bST × day of blood sampling interaction.

[7]bST × time after feeding interaction.

[8]bST × day × time interaction significant ($P < .01$).

[9]Day × time interaction significant ($P < .01$).

by bST supplementation or day of sampling (3 vs. 10 after the third bST injection).

Concentration of NEFA was significantly increased by bST at d 10, but not at d 3. Increase at d 10 was observed from −70 to 50 min after feeding ($P < .01$) and at 70 and 90 min after feeding ($P < .05$), but not thereafter. Consequently, there were significant interactions (bST × day of sampling, bST × time after feeding, and bST × day × time; Table 3). Uremia was not significantly ($P > .22$) decreased by bST supplementation. Interaction of day of sampling × time after feeding was significant.

## DISCUSSION

### Effects of bST Treatment

*Plasma bST.* The pulsatile pattern of endogenous plasma bST in the control group agrees with others (22, 31). The number of spikes (approximately 4 per 6-h period) is similar to those (12 to 16 per 24 h) found in lactating cows (22), growing steers (11), and young calves (10). A smaller number of spikes (8 to 10 per 24 h), however, sometimes has been reported in lactating cows (31) and growing steers (5). There was no clear effect of feeding time on plasma bST [(31) and present study in control cows; Figure 2].

The pulsatile pattern observed in bST-supplemented cows (Figure 3) was unexpected because there is no known reason for thinking that an episodic release of bST can occur from the injection site. Moreover, there is an expected negative feedback of exogenous bST on pituitary endogenous secretion. Our bST assay did not allow for distinction between plasma endogenous hormone and exogenous hormone. It is possible that the pituitary of the treated cows still episodically released bST after only three injections (R. J. Collier, personal communication), but areas of endogenous spikes (Figure 2) were too small, if they occurred, to explain the large spike areas in bST cows (Figure 3). It also can be hypothesized that bST clearance rate was not constant during the day, although experimental proofs are not available. Our results are not in accordance with those of Schams et al. (24), who reported that bST supplementation only increased plasma bST concentrations to a higher plateau.

The mean plasma bST concentration in supplemented cows was much greater than in controls but fell within the range observed in growing cattle (24). It was higher at d 10 than at d 3 after the third injection, in accordance with

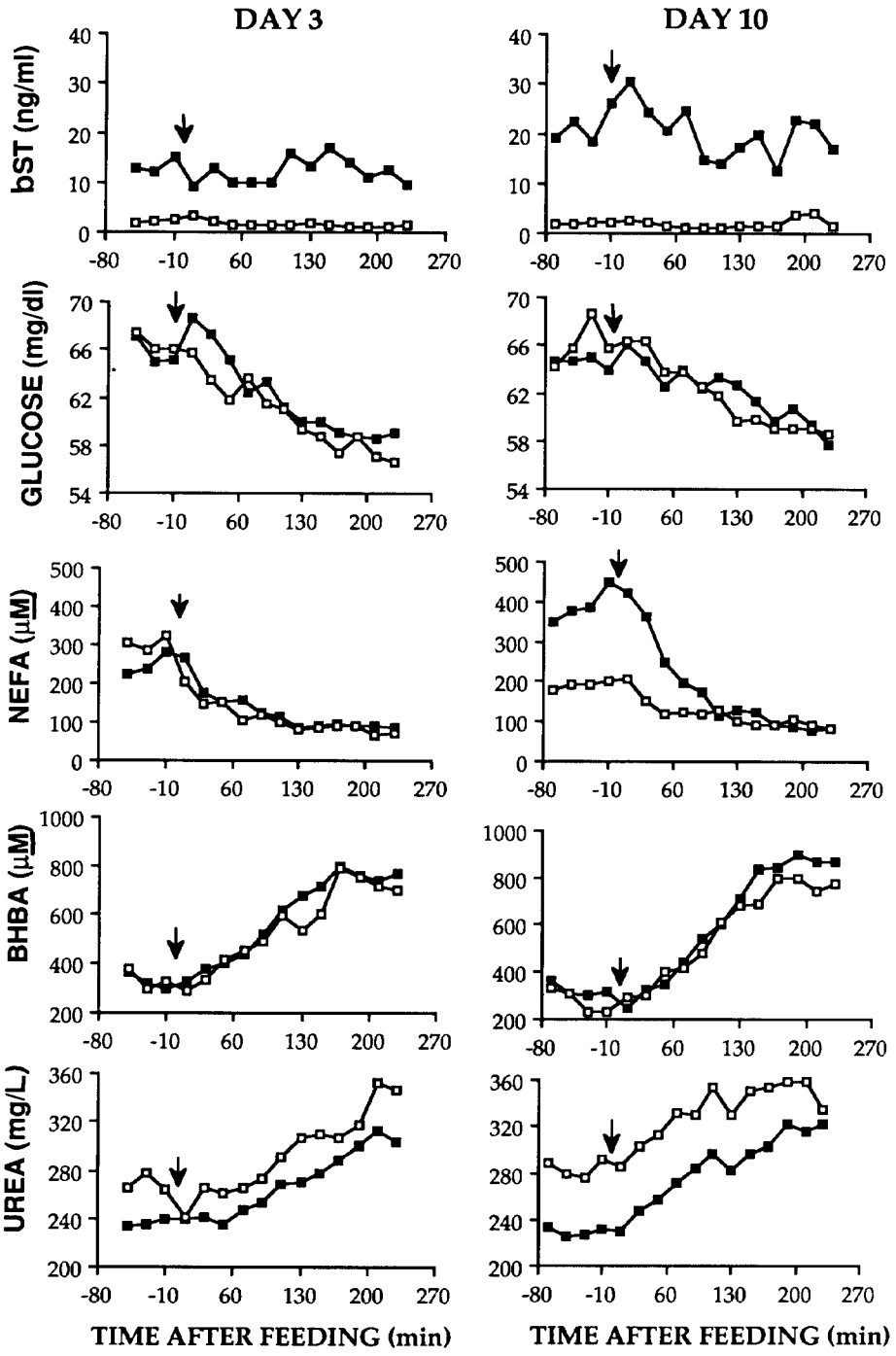


Figure 4. Mean plasma values of bST and metabolites at d 3 and 10 after the third bST (7 cows, ■) or placebo (5 cows, □) injection (arrows indicate time of feeding; BHBA = beta-hydroxybutyrate).

results obtained after injection of a similar dose (640 mg) of another slow release preparation (Somidobove; Elanco, Indianapolis, IN) (32), and occurred simultaneously with maximum milk yield response around d 10 (20, 32). However, in other studies with either sometribove or somidobove (24, 27), plasma bST concentrations were maximum during the first 5 d. With daily injections of bST, plasma bST was higher at d 10 than at d 1 in one trial (18), but not in another (16). New data on bST entry and clearance rates are needed to better understand fluctuations of plasma bST after injections of slow release preparations.

*Plasma IGF-I.* Plasma IGF-I concentration was greatly increased by bST supplementation (Table 1) as reported by others (15, 34). Some evidence suggests a role for IGF-I in mediating mammary gland stimulation in bST-supplemented cows. Interpretation of plasma IGF-I concentration, however, is complicated because most of the circulating IGF-I is bound to specific binding proteins, thus attenuating its biological activity (19). The positive relationship between plasma IGF-I and energy balance in control cows (Figure 1) agrees with published data (5, 11). Moreover, the rise in serum IGF-I during bST supplementation was decreased by protein or energy deficiencies in cows (21, 34) and steers (5, 11). In our trial, the correlation between plasma IGF-I and energy balance in bST cows was only slightly positive, but most energy balances were positive, and the range of variation was small (Figure 1). Modulation of somatotropin effects on IGF-I secretion at the hepatic receptor level by insulin, $T_3$, or other anabolic hormones has been postulated (29). Insulin and $T_3$ were correlated positively to energy balance in our trial ($r = .32$ and .51, respectively, $n = 200$).

*Plasma Insulin, $T_3$, and Metabolites.* Plasma glucose, BHBA, and $T_3$ concentrations were not affected by bST supplementation, in accordance with others (12, 19, 28). A tendency for increased insulinemia has sometimes, but not always, been observed during bST supplementation (12, 19, 28). It also was reported that bST induced a peripheral resistance to insulin (15), although the decline of plasma NEFA after feeding (Figure 4) suggests no loss of insulin antilipolytic effect. Using insulin challenge in vivo, Sechen et al. (26) even observed an increased antilipolytic effect in bST-supplemented cows.

A direct lipolytic effect of pituitary somatotropin has been suggested earlier by in vivo and in vitro studies but was due in part to contamination by other lipolytic hormones. Most, but not all, recent studies with highly purified pituitary or recombinant somatotropin have failed to show a direct lipolytic effect (33).

Somatotropin, however, can alter the responsiveness of adipose tissue to other lipolytic hormones. In vivo, it increased the response to catecholamine challenge in lactating cows (13, 26), although adipose tissue from bST-supplemented steers (17) or lactating cows (27) did not respond more to catecholamines in vitro, even when the in vivo response was higher (17). It also was found that in vitro exposure of sheep adipose tissue to somatotropin increased both the number of beta-adrenergic receptors and the sensitivity of lipolysis to a beta-agonist, isoproterenol (33). Lipolysis also can be stimulated by decreasing antilipolytic responses. This was the case in sheep [decrease in α-2-adrenergic antilipolytic responses after bST addition in vitro; (33)].

Higher blood concentrations of NEFA indicating adipose tissue mobilization in bST cows generally were observed during the first months of bST supplementation, when energy balance was decreased [(2, 28) and the present study at wk 14; Table 1]. However, there was no increase in plasma NEFA concentration in short-term trials or after several weeks of bST supplementation in long-term trials, when energy balance was positive [(15, 25) and the present study at wk 20; Table 1]. The increase we observed in bST cows at wk 14 was, however, partly independent of energy balance, because it remained (71% higher than in controls; $P < .01$) when energy balance was taken into account as covariate ($r = -.48$; between energy balance and plasma NEFA; $n = 50$). A decrease in body lipids was often observed in bST-supplemented cows (7, 28), and this was confirmed in the present trial (9).

Plasma NEFA were not increased at d 3 after the third bST injection but only before and shortly after feeding at d 10. Milk yield response, milk fat content (20), and circulating bST (Figure 4) all were higher at d 10. These results confirm that bST acts as a teleophoretic

or homeorhetic hormone by altering the responses to homeostatic regulations involved in hour to hour and day to day metabolic adaptations to productive and nutritional status. Interactions between bST and excitement around feeding were observed in growing heifers (4), but this probably did not operate in our study as shown by d 3 results (Figure 4).

The significant decline in uremia at wk 20 (Table 1) agrees with previous results (12) and is consistent with capacity of bST to spare amino acids from catabolism and decrease urinary N excretion (25). In the present study, bST effect on uremia was higher, although not significantly so, at d 10 than at d 3 after the third bST injection (Figure 4 and Table 3), i.e., when milk yield response and circulating bST were higher. Furthermore, the significant effect at wk 20 seemed to be related to a decrease in protein balance from wk 14 (Table 1). At wk 20, uremia was related positively to protein balance ($r = .46$; $n = 50$), and estimated bST effect on uremia was lower (–30 instead of –47 mg/L; Table 1) when protein balance was used as covariate. Consequently, there were two effects on uremia: a positive effect of protein balance and a negative effect of bST (for the same protein balance).

### Effects of Parity

The significantly lower concentrations of plasma $T_3$ in multiparous versus primiparous cows (Table 1) probably was due in part to the larger milk yield of the former, because thyroid hormones are excreted by the mammary gland, or due to an effect of age per se (3). A lower concentration of plasma $T_3$ also was reported to be associated with lower energy balance (3). This probably was not the case in our study, because calculated energy balance was not different between primiparous and multiparous cows and because the true energy balance would have been lower in primiparous cows if requirements for growth had been taken into account in computations. Effects of $T_3$ in modulating IGF-I production (29) might also account for the significantly higher plasma IGF-I in primiparous cows at wk 14. However, the bST × parity interaction at wk 20 (less increase in primiparous cows) did not confirm this hypothesis, because $T_3$ was higher in primiparous cows.

Primiparous cows showed a significant increase in plasma NEFA above multiparous animals (Table 1). This might be related to their better condition score before bST supplementation (20), resulting in a greater capacity for fat mobilization, and also to the higher $T_3$ level in their plasma, possibly allowing increased lipolysis (30). This can furthermore be related to the additional requirements of nutrients for growth. There were, however, no significant interactions between bST and parity either on plasma NEFA and $T_3$ (Table 1) or on change in condition score during wk 9 to 20 (20).

### Effects of Concentrate Level

Cows given low amounts of concentrate ate more corn silage, resulting in no significant difference in energy intake between nutritional groups (20). This might in part explain the lack of significant interactions between bST supplementation and the level of concentrate on measured traits. The significant increase in plasma BHBA in the low concentrate group (Table 1) was probably from exogenous source, resulting from the higher corn silage intake (20) rather than from endogenous origin related to a lower energy balance, because there was no difference in plasma NEFA concentration.

In conclusion, administration of bST did not significantly affect plasma concentrations of glucose, BHBA, insulin, and $T_3$ but significantly altered plasma bST, IGF-I, NEFA, and urea. The rise of NEFA, when it occurred, probably was due both to a bST effect on energy balance (decrease) and to interactions of bST with short-term homeostatic regulations. In the same way, bST-induced decline in uremia seemed to be related both to decrease in protein balance and to bST per se. These coordinated teleophoretic (or homeorhetic) modifications during bST supplementation allowed a rise in milk yield without disturbing homeostasis, as reflected by plasma hormone and metabolite concentrations.

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