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SOMATOMEDIN C IN DAIRY COWS RELATED TO ENERGY AND PROTEIN SUPPLY AND TO MILK PRODUCTION

H. RONGE[1], J. BLUM[1]†, C. CLEMENT[1], F. JANS[2], H. LEUENBERGER[3] AND H. BINDER[4]

[1]*Department of Nutrition Pathology, Institute of Animal Breeding, University of Berne, School of Veterinary Medicine, 3012 Berne, Switzerland*

[2]*Federal Research Station for Animal Production, Grangeneuve, 1725 Posieux, Switzerland*

[3]*Institute of Animal Science, Federal Institute of Technology, 8092 Zurich, Switzerland*

[4]*Institute of Animal Breeding, University of Zurich, School of Veterinary Medicine, 8057 Zurich, Switzerland*

ABSTRACT

Somatomedin C and other hormones, as well as blood metabolites, were measured during the dry period and during lactation in dairy cows, given different amounts of energy and protein, to study metabolic and endocrine adaptations. Somatomedin C, specifically measured by radioimmunoassay after separation from its binding protein, did not exhibit typical diurnal variations, in contrast to somatotropin and insulin, which increased particularly after concentrate intake. Somatomedin C markedly decreased at parturition and reached lowest values around the peak of lactation, while levels of somatotropin, non-esterified fatty acids and ketone bodies were high and those of glucose, insulin, thyroxine and triiodothyronine were low. Thereafter somatomedin C values slowly increased up to the 12th week of lactation and remained elevated. Low energy and protein balances were characterized by particularly low somatomedin C concentrations. An additional protein deficit at peak lactation, when cows were already provided with low amounts of energy, did not further decrease somatomedin C levels. However, when high amounts of energy were given in the form of starch or crystalline fat, somatomedin C increased. Overall, there was a positive correlation of somatomedin C primarily with energy, but also with protein balances and a negative correlation with milk yield. Conversely, somatotropin increased markedly after parturition and was positively correlated with milk production and negatively with protein and energy balances. Thus, somatomedin C levels were paradoxically low in the presence of high circulating somatotropin. Insulin most closely paralleled somatomedin C levels. Therefore the anabolic state of metabolism at the end of pregnancy was characterized by high somatomedin C and insulin and relatively low somatotropin, whereas the catabolic state of early lactation was characterized by high somatotropin, low somatomedin C, insulin and thyroid hormones.

INTRODUCTION

HIGH-yielding dairy cows are usually characterized by negative energy and protein balances during the first weeks of lactation (Moe, 1981; Chilliard, Rémond, Sauvant and Vermorel, 1983). During early lactation metabolism is changed towards enhanced mobilization and utilization of fat whereas glucose utilization in organs other than the mammary gland is reduced (Bennink, Mellenberger, Frobish and Bauman, 1972; Bauman and Currie, 1980; Rémésy, Chilliard, Rayssiguier, Mazur and Démigné, 1986; Chilliard, 1987). Marked endocrine changes occur that are considered responsible for the shift from a largely anabolic metabolic state during late pregnancy towards a primarily catabolic state in early lactation (Bauman and Currie, 1980; Tucker, 1981; Hart, 1983; Collier, McNamara, Wallace and Dehoff, 1984; Kunz and Blum, 1985; Kunz, Blum, Hart, Bickel and Landis, 1985; Karg and Mayer, 1987).

Somatotropin (STH), circulating in blood in relatively high amounts particularly during early lactation, decreases lipogenesis, favours

† To whom correspondence should be addressed.

lipolysis and gluconeogenesis, enhances nitrogen retention and stimulates milk yield (Hart and Johnsson, 1986; McCutcheon and Bauman, 1986), although receptors for STH are not present in mammary tissue (Tucker and Merkel, 1987). However, several of the effects of STH are well known to be mediated by somatomedin C (SmC). Importantly, receptors for SmC exist in mammary tissue (Campbell and Baumrucker, 1986). Therefore SmC might be responsible for STH effects on mammary tissue and could mediate, at least in part, STH effects on milk yield. Bovine SmC is identical with human SmC and with insulin-like growth factor I (Klapper, Svoboda and Wyk, 1983; Honegger and Humbel, 1986). It seems mainly to be synthesized by the liver.

Because blood SmC levels are modified in rats, humans, steers and heifers by variations in protein and/or energy intake (Breier, Bass, Butler and Gluckman, 1986; Elsasser, Rumsey and Hammond, 1986; Underwood, Clemmons, Maes, D'Ercole and Ketelslegers, 1986) and somatomedin-like activity (determined by bioassay) is reduced in lactating cows with insufficient energy intake or high milk production (Falconer, Forbes, Bines, Roy and Hart, 1980; Binnerts, Adrichem, Oudenaarden, Vogt and Wassenaar, 1982), we have studied changes of SmC levels by use of a specific radioimmunoassay in high-yielding dairy cows, given different amounts of energy and/or protein.

MATERIAL AND METHODS

Experimental design and feeding

Four studies (A, B, C and D) were performed: over an entire lactation (A), under different feeding conditions from 2 weeks before to 12 weeks after parturition (B and C), and as a 24-h experiment during the 4th or 5th week of lactation under different feeding conditions (D).

Experiment A

Animals. Ten dairy cows (Schweizerisches Braunvieh) in their second lactation were examined for 300 days. Three of them were non-pregnant whereas the other seven were successfully inseminated between the 8th and 20th week of lactation.

Feeding. The animals were fed individually and received hay *ad libitum* and 2 kg barley. When they produced more than 13 kg milk, concentrates (1 kg/2 kg milk; containing cereals, soya-bean meal, vitamins and minerals) were fed in addition. The experiment was performed and the animals were held at the Institute for Animal Breeding at the University of Zurich (Binder, 1986). Blood samples were taken every 2nd day from 2 to 80 days, and then every 5th day up to 300 days.

Experiment B

Animals. Thirty dairy cows (16 Holstein-Friesian, eight Simmental and six Schweizerisches Braunvieh × Brown Swiss) were divided equally by breed and numbers of lactation into two groups, each consisting of 15 animals: group HE was fed high amounts of energy and protein using concentrates; group LE was fed low amounts of energy and protein by omitting the concentrates.

The animals were held at the Research Station of the Institute of Animal Science, Federal Insititute of Technology, Zurich (Leuenberger, Kunz and Michel, 1987). Blood samples were taken once weekly.

Feeding. Both groups were fed individually a ration of 2/3 grass or hay and 1/3 maize silage. Group HE received concentrates (450 g barley, 200 g wheat, 150 g oats, 175 g maize per kg and vitamins and minerals; supplemented with a soya-bean meal).

Experiment C

Animals. Nineteen animals (one animal had to be treated for ketosis and was eliminated from the experiment) (12 Simmental × Red Holstein, and seven Brown Swiss × Schweizerisches Braunvieh) were divided equally by breed into two groups: group LD was fed normally (according to recommendations), and had a low protein deficit (10 animals); group HD received less protein and therefore had a high protein deficit (nine animals).

Feeding. To 5 kg hay, concentrates (315 g barley, 315 g maize, 150 g soya-bean meal,

117 g oats per kg and molasses, minerals and vitamins; group LD additionally received soya-bean meal) and maize silage were given according to the planned deficit of each animal. In group HD an additional protein deficit was planned. Planned daily energy and protein balances, based on net energy lactation (NEL) and absorbable protein from the gut (AP) for the 1st, 2nd and 3rd month were: for LD −22·5, −12·5 and 0 MJ NEL and −125, −25 and 0 g AP; for HD −32·5, −20 and 0 MJ NEL and −500, −325 and 0 g AP. The animals were held and individually fed at the Federal Research Station for Animal Production in Grangeneuve (F. Jans, unpublished). Blood samples were taken once weekly.

Experiment D

Animals. Eighteen dairy cows (nine Simmental × Red Holstein, and nine Schweizerisches Braunvieh purebred or × Brown Swiss) were divided equally by breed into three different groups of six animals each: group LE was given low amounts of energy; group HE received relatively high amounts of energy; group CF received concentrates supplemented with crystalline fat.

Feeding. The animals received hay and maize silage *ad libitum*. Concentrates (soya-bean meal supplemented with barley, maize, minerals and vitamins) were added as planned. Group CF received 1150 g crystalline fat per day (Alikon ®; Alifet AG, 4922 Bützberg, Switzerland).

Experiments in the 4th to 5th week of lactation, lasting for 7 days, were carried out at the Swiss Federal Research Station for Animal Production in Grangeneuve (F. Jans, unpublished). Blood samples (39) were taken over a 24-h period (Blum, Jans, Moses, Fröhli, Zemp, Wanner, Hart, Thun and Keller, 1985a) at the last day of the experiment.

In experiments B, C and D, energy and protein intake and balances were based on NEL and AP, respectively, and were calculated according to Bickel and Landis (1978) and Landis (1979). Calculations of AP balances were modified by taking milk protein instead of fat-corrected milk (FCM) as a basis (1·5 g AP for 1 g milk protein). Calculations of daily requirements were based on:

maintenance: 0·293 MJ NEL per kg $M^{0.75}$ and 3·25 g AP per kg $M^{0.75}$
pregnancy (9th month): 18 MJ NEL and 205 g AP
milk: 3·14 MJ NEL per kg FCM and 1·5 g AP per g milk protein.

In experiment A, protein intake was measured based on digestible crude protein.

Blood samples were taken at 14.00 h by jugular venipuncture, using vacutainers (Becton-Dickinson, CH 4142 Müchenstein) or through indwelling catheters (24-h experiments, D), implanted at least 3 h before the start of the studies. Heparinized blood plasma was used for all determinations, except for the determination of ketone bodies, for which blood was deproteinized with equal amounts of ice-cold perchloric acid (0·7 mol/l).

Laboratory methods

Glucose, protein, albumin, urea, non-esterified fatty acids (NEFA), ketone bodies (β-hydroxybutyrate plus acetoacetate), immunoreactive insulin (IRI), thyroxine (T_4) and triiodothyronine (T_3) were measured as described before (Blum *et al.*, 1985a).

STH concentrations were determined by radioimmunoassay based on the methods of Hart, Flux, Andrews and McNeilly (1975) with some modifications. STH for standards (USDA-bGH-B-1 AFP-5200) and for iodination (USDA-bGH-I-1 AFP-6500) were obtained from the National Institute of Arthritis, Diabetes and Digestive and Kidney Diseases, Baltimore, MD 21201-3472, USA. Antiserum against bovine rbSTH (bGH 5/15.6.86), raised in a rabbit, was provided by Professor Dr D. Schams, Munich and used at a final dilution of 1:50000. Goat-anti-rabbit-gamma-globulin, purchased from Antibodies Inc., Davis, CA, USA, fraction P_4, was used as second antibody to separate (together with 5% polyethyleneglycol) the bound from free hormone. STH was labelled using the iodogen procedure (Salacinski, McLean, Sykes, Clement-Jones and Lowry, 1981). rbSTH paralleled bSTH standards obtained from hypothalamic extracts. Half-maximal binding was attained with 10 μg STH

per l and the sensitivity was below 1 μg/l. Recovery of bovine STH for 1 and 10 ng added (10 μl) to 1 ml bovine serum was 117 ± 17% and 107 ± 6%, respectively. All samples from one animal were measured within the same assay and each sample was determined in triplicate. Intra-assay coefficient of variation and inter-assay variation were below 0·050.

Somatomedin C was determined in triplicate by radioimmunoassay according to Zapf, Walter and Froesch (1981) with some modifications. SmC (preparation 1/3 and 1/4) used for standards was kindly provided by Professor Dr R. E. Humbel, Zürich, Switzerland. For production of antiserum in a rabbit and for iodination (by the chloramin-T method), recombinant human SmC (rSmC, Mü 14 Fr 25-32 TOP), which has the same structure as bovine SmC or insulin-like growth factor I, was used (rSmC was obtained from Professor Dr Nüesch and Dr Scheibli, Ciba-Geigy A. G., 4002, Basle, Switzerland). All samples were pretreated with acid/ethanol to separate SmC from its binding protein(s), as described by Daughaday, Mariz and Blethen (1980). The samples were neutralized with ammonium hydrogen carbonate, lyophilized and reconstituted in the assay buffer before further use. After incubation for 24 h with antibody and another 24 h with tracer, antibody-bound and free SmC were separated after addition of 1% bovine gamma-globulin and 25% polyethyleneglycol by centrifugation. Half-maximal binding was 1·0 ng per tube. The sensitivity was below 0·1 ng per tube (less than 6·5 μg/l). Recovery was 91 ± 5% and 111 ± 9%, respectively, for 10 and 20 ng rSmC (10 μl) added to 1 ml bovine plasma. The recovery of ^{125}I-labelled rSmC added to bovine plasma was 104 ± 1%. Compared with separation of SmC bound to plasmaprotein(s) by chromatography using a large column (30 × 2 cm, volume 92·5 ml) and acidified buffer, recovery by acid/ethanol extraction was increased by 27 ± 5%. Diluted sera from cattle in different physiological states paralleled the standard curve. Heparin in plasma did not modify the results. Use of an antiserum (115/91177, obtained from Professor J. Zapf, University of Zürich, Switzerland) raised against extracted human SmC gave the same results as obtained with the antiserum raised against rSmC. The intra-assay coefficient of variation and the inter-assay coefficient of variation were below 0·1.

Statistical analysis

Values are expressed as means and standard errors. Correlations were calculated according to Spearman. They were calculated for each animal separately and are expressed as the mean of individuals. The significance of difference between groups were tested by Wilcoxon-Test ($P < 0·05$).

RESULTS

Changes of SmC and milk yield during a 300-day lactation period (experiment A)

The day-to-day coefficient of variation was 0·14 and 0·17 for two animals during the first 80 days of lactation (Figure 1). Following a transient decrease after parturition, SmC levels increased up to 150 days of lactation and then remained elevated. In contrast, milk production (FCM) continuously decreased. There were no significant differences between pregnant and non-pregnant cows (not shown). Energy and protein balances never became negative throughout lactation and increased during the first 2 to 3 months after parturition. SmC and FCM were negatively correlated ($r = -0·61$), whereas there was a positive correlation of SmC with energy and protein balances ($r = 0·42$ and 0·43, respectively).

Effects of energy and protein supply on food intake, energy and protein balances, milk yield and composition, metabolites and hormones (SmC, STH, IRI, T_3 and T_4) during the dry period and the first 3 months of lactation (experiments B and C)

Dry-matter intake of basic ration (BR; grass, hay, maize silage) was relatively low during the dry period, then increased particularly during the 1st and 2nd month of lactation, but there were no significant differences between groups LE and HE or LD and HD, respectively (Table 1). Provision

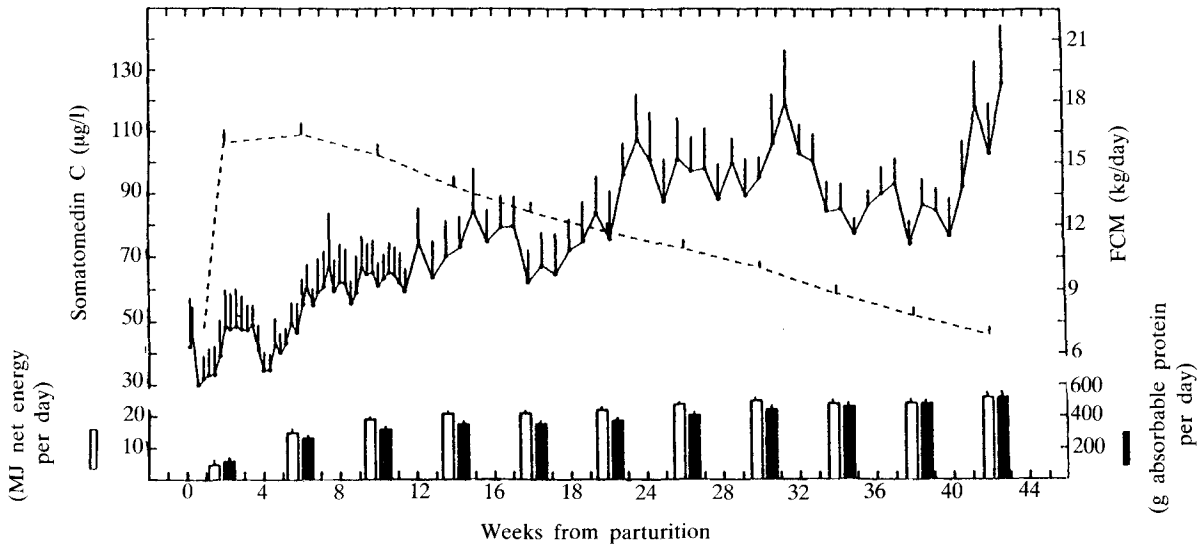

FIG. 1. Blood levels of somatomedin C measured every 2nd day from 2 to 80 days, and every 5th day up to 300 days; ± s.e. (———), fat-corrected milk yield (FCM) (- - -), energy (MJ net energy per day □) and protein (g absorbable protein per day ■) balances (average calculated for 4 weeks; during a 300-day lactation period (experiment A).

of concentrates (C) was increased during lactation compared with the dry period. Concentrates were not supplied in experiment B to animals of group LE. Concentrate intake in experiment C was lower for group HD, but this difference was not significant.

NEL intake was relatively low during the dry period and continuously increased after parturition during the 3 months of the study, but there were significant differences between the groups only in experiment B (Table 1; Figure 2). NEL balances were positive before, but negative after parturition, particularly in early lactation. They were more negative for group LE than for group HE and tended to be lower for group HD than for group LD ($P < 0{\cdot}05$ for the 1st month of lactation).

AP intake was relatively low during the dry period. It increased during lactation, in groups HE and LD particularly during the 1st month and there were signficant differences ($P < 0{\cdot}05$) between groups LE and HE, as well as between LD and HD (Table 1). AP balances were positive before parturition and became transiently negative during the 1st month of lactation in groups LE and LD and during the first 8 weeks of lactation in group HD. AP balances were significantly lower in group LE throughout the study than in group HE and in group HD during the first 8 weeks *post partum* compared with group LD ($P < 0{\cdot}05$).

Milk yield (FCM) increased rapidly during the first 2 to 4 weeks and was maximal during the 2nd month of lactation. It was significantly higher during the study in group HE than LE ($P < 0{\cdot}05$ for the 2nd and 3rd month of lactation), but comparable in groups LD and HD. Milk fat content decreased during the 1st month of lactation, but was not significantly different between experimental groups. Milk protein content transiently decreased during the 2nd month of lactation, but was lower in group LE than in group HE ($P < 0{\cdot}05$ during the 2nd and 3rd month of lactation) and in group HD than in group LD (not significantly). Lactose content was numerically lower during the 1st month of lactation, but there were no significant differences between the groups.

Plasma concentrations of glucose transiently decreased during the 1st month of lactation (Table 2; Figure 3). Glucose levels were significantly lower during the first 2 months of lactation in group LE compared with group HE ($P < 0{\cdot}05$). NEFA concentration reversibly increased during the 1st month of

lactation, particularly in group LE, with significant differences between groups HE and LE for the first 2 months of lactation. Ketone bodies (acetoacetate plus β-hydroxybutyrate) increased after parturition. The increase was highest and significantly greater in group LE than in group HE ($P < 0{\cdot}05$ for the first 2 months of lactation), but only numerically greater for group HD than for group LD.

Plasma concentrations of protein were higher during lactation than during the dry period in experiment B, but not in experiment C and levels were significantly higher in group HE than in group LE during the whole study (Table 2, $P < 0{\cdot}05$). Albumin was increased during lactation compared with the dry period. Levels were significantly lower in group LE than in group HE for the first 2 months of lactation ($P < 0{\cdot}05$). Urea transiently decreased in group LE and in group HD ($P < 0{\cdot}05$), but increased in groups HE and LD ($P < 0{\cdot}05$) during lactation. Levels were lower in group LE than in group HE ($P < 0{\cdot}05$) and in group HD than in group LD ($P < 0{\cdot}05$ for the first 8 weeks after parturition).

Levels of SmC were highest during the dry period (Table 2; Figure 4). SmC sharply decreased at parturition and remained lowest during the 1st month of lactation in all experimental groups and then slowly increased again. SmC concentrations were always lower

TABLE 1

Dry matter (DM) (basic ration (BR) and concentrates (C)), net energy lactation (NEL) and crude protein (CP) intakes and absorbable protein (AP) intakes and balances, fat-corrected milk yield (FCM) and milk composition during the dry period (last 2 weeks before parturition) and during the first 3 months of lactation.

		Experiment B							
				Weeks of lactation					
		Dry period		1 to 4		5 to 8		9 to 12	
No of animals	HE	15		15		15		15	
	LE	15		15		15		15	
		Mean	s.e.	Mean	s.e.	Mean	s.e.	Mean	s.e.
Body weight	HE	711	11^{a}	640	10^{b}	617	10^{c}	614	11^{c}
(kg)	LE	681	19^{a}	592*	18^{b}	566*	16^{c}	562*	16^{d}
DM intake	HE	12·7	0.5^{a}	15·1	$0{\cdot}6^{b}$	17·9	0.6^{c}	18·3	$0{\cdot}5^{c}$
BR (kg/day)	LE	13·2	$0{\cdot}9^{a}$	15·7	$0{\cdot}6^{b}$	17·6	$0{\cdot}7^{c}$	18·9	$0{\cdot}6^{c}$
DM intake	HE	2·6	$0{\cdot}1^{a}$	3·7	$0{\cdot}3^{b}$	3·3	$0{\cdot}3^{b}$	2·6	$0{\cdot}4^{ab}$
C (kg/day)	LE	0*	0	0*	0	0*	0	0*	0
NEL intake	HE	93	3^{a}	117	2^{b}	132	2^{c}	132	3^{c}
(MJ/day)	LE	75*	4^{a}	92*	3^{b}	105*	4^{c}	115*	4^{c}
NEL balance	HE	34	3^{a}	−7	3^{b}	0	3^{b}	7	1^{c}
(MJ/day)	LE	18*	4^{a}	−23*	3^{b}	−10	2^{c}	4	2^{d}
CP intake	HE	2083	64^{a}	3514	86^{bd}	3643	96^{bc}	3278	94^{d}
(g/day)	LE	1735*	94^{a}	1984*	71^{ab}	2101*	81^{b}	2630*	69^{c}
AP intake	HE	1480	45^{a}	2171	43^{b}	2419	53^{c}	2253	63^{b}
(g/day)	LE	1167*	70^{a}	1392*	48^{b}	1548*	61^{c}	1776*	58^{d}
AP balance	HE	828	44^{a}	400	39^{b}	613	27^{c}	518	44^{d}
(g/day)	LE	529*	66^{a}	−209*	39^{b}	49*	28^{c}	301*	33^{bc}
FCM	HE			27·0	1·2	30·6	1·3	28·1	1·1
(kg/day)	LE			24·9	1·0	25·7*	1·0	24·5*	1·1
Milk fat	HE			42·4	1·3	40·0	1·1	40·2	1·0
(g/l)	LE			44·2	$1{\cdot}3^{a}$	40·4	$0{\cdot}6^{b}$	40·3	$0{\cdot}8^{b}$
Milk protein	HE			34·0	$0{\cdot}8^{a}$	30·5	$0{\cdot}5^{b}$	31·6	$0{\cdot}5^{b}$
(g/l)	LE			33·7	$0{\cdot}6^{a}$	29·1*	$0{\cdot}3^{b}$	29·9*	$0{\cdot}3^{c}$
Milk lactose	HE			49·6	0·4	50·8	0·4	50·5	0·3
(g/l)	LE			48·9	0·4	50·0	0·4	49·8	0·3

		Experiment C							
				Weeks of lactation					
		Dry period		1 to 4		5 to 8		9 to 12	
No of animals	LD	10		10		10		10	
	HD	9		9		9		9	
		Mean	s.e.	Mean	s.e.	Mean	s.e.	Mean	s.e.
Body weight	LD	742	18^{a}	671	18^{b}	663	17^{b}	666	18^{b}
(kg)	HD	791	16^{a}	707	12^{b}	698	14^{b}	694	14^{b}
DM intake	LD	11·5	$0·4^{a}$	12·8	$0·4^{b}$	13·5	$0·5^{b}$	13·7	$0·4^{ab}$
BR (kg/day)	HD	11·8	$0·4^{a}$	13·2	$0·6^{ab}$	14·2	$0·6^{b}$	13·8	$0·4^{b}$
DM intake	LD	1·3	$0·1^{a}$	4·2	$0·4^{b}$	6·5	$0·9^{b}$	6·7	$0·9^{b}$
C (kg/day)	HD	1·3	$0·1^{a}$	3·2	$0·4^{b}$	5·0	$0·7^{bc}$	6·5	$0·8^{c}$
NEL intake	LD	79	3^{a}	109	2^{b}	132	2^{c}	136	3^{c}
(MJ/day)	HD	81	2^{a}	104	2^{b}	125	2^{c}	134	3^{c}
NEL balance	LD	20	3^{a}	−25	2^{b}	−15	2^{c}	−2	1^{d}
(MJ/day)	HD	19	2^{a}	−32*	2^{b}	−18	2^{c}	−5	1^{d}
CP intake	LD	1585	70^{a}	2590	92^{b}	3029	143^{c}	2671	135^{bc}
(g/day)	HD	1618	37^{a}	2099*	46^{b}	2444*	89^{c}	2656*	102^{c}
AP intake	LD	1200	50^{a}	1843	51^{b}	2188	29^{c}	2052	42^{d}
(g/day)	HD	1233*	26^{a}	1588*	20^{b}	1876*	47^{c}	2030	49^{c}
AP balance	LD	534	45^{a}	−155	17^{b}	128	19^{c}	95	18^{c}
(g/day)	HD	545	28^{a}	−342*	29^{b}	−71*	10^{c}	110	17^{d}
FCM	LD			30·4	$1·3^{a}$	34·5	$0·6^{b}$	31·6	$0·7^{a}$
(kg/day)	HD			30·7	0·9	33·0	1·1	31·6	1·1
Milk fat	LD			42·7	$1·4^{a}$	40·2	$0·9^{b}$	40·9	$1·0^{ab}$
(g/l)	HD			42·6	1·6	41·1	1·8	41·4	2·1
Milk protein	LD			36·2	$0·9^{a}$	31·6	$0·4^{b}$	32·7	$0·7^{b}$
(g/l)	HD			33·6	$0·7^{a}$	30·6	$0·6^{b}$	31·5	$0·7^{ab}$
Milk lactose	LD			49·2	0·5	50·5	0·3	49·7	0·3
(g/l)	HD			49·4	0·7	50·6	0·3	50·3	0·4

[a,b,c] Different superscripts indicate significant differences between dry period, week 1 to 4, week 5 to 8 or 9 to 12 of lactation ($P < 0·05$).

* Significant difference between groups LE and HE and LD and HD ($P < 0·05$).

in group LE than in group HE ($P < 0·05$ for the dry period and the 1st month of lactation), but similar in groups LD and HD.

In contrast to SmC, STH increased after parturition, with highest levels reached during the 1st month of lactation ($P < 0·05$). STH was significantly higher in group LE than in group HE during lactation ($P < 0·05$), but similar in groups LD and HD.

Levels of IRI, as those of SmC, were highest during the dry period and rapidly decreased after parturition, remained low during the 1st month of lactation and then increased again. IRI levels were lower in group LE than in group HE ($P < 0·05$ during the first 2 months of lactation), but similar in groups HD and LD.

Concentrations of T_3 transiently decreased after parturition in experiment B, particularly in group LE, while they increased continuously in experiment C. Levels of T_3 were always lower in group LE than group HE (not significantly), but comparable in groups LD and HD. T_4 levels transiently decreased in all groups after parturition, but there were significant differences only for the 1st month of lactation between groups HE and LE.

SmC showed highest positive correlations ($P < 0·05$) with energy (Figure 5) and protein balances, with glucose, IRI, T_4 and T_3 in both experiments (Table 3). Negative energy balances were related to low concentrations of SmC. Elevated SmC values were found if energy balances were positive. Correlations with AP balances were higher in experiment C. Energy and protein balances were closely related in both experiments ($r = 0·83$), but

partial correlations revealed only negligible effects of AP balance (not shown). There were negative correlations of SmC with FCM, ketone bodies and STH.

Effects of different energy and protein intake and of crystalline fat on energy and protein balances, milk yield and composition, metabolites and hormones (SmC, STH, IRI, T_3 and T_4) during a 24-h period (experiment D)

Levels of SmC did not change during the 24-h period (Figure 6). In contrast, IRI and STH exhibited marked diurnal variations, mainly due to an increase in response to concentrate feeding (at 06.00 h and 14.30 h).

Body weight tended to be higher in groups HE and CF than in group LE (Table 4). Intake of BR was similar, whereas C, NEL and AP intake were lowest in group LE and highest in group CF. NEL and AP balances were most markedly negative in group LE, but positive in group CF.

FCM and milk lactose were similar in groups LE, HE and CF, whereas milk fat and protein were highest in group LE and lowest in group CF ($P < 0.05$).

Glucose levels were lowest in group LE and highest in group CF, whereas the reverse was found for NEFA, ketone bodies and urea, while protein and albumin levels were not consistently changed.

SmC, IRI, T_3 and T_4 were numerically or significantly lower in group LE than in groups HE or CF. In contrast, STH was highest in group LE and lowest in group CF.

TABLE 2

Blood metabolites and hormones during the dry period (last 2 weeks before parturition) and during the first 3 months of lactation

		Experiment B							
				Weeks of lactation					
		Dry period		1 to 4		5 to 8		9 to 12	
		Mean	s.e.	Mean	s.e.	Mean	s.e.	Mean	s.e.
Glucose	HE	3·25	$0·09^{a}$	3·02	$0·10^{b}$	3·12	$0·07^{ab}$	3·19	$0·08^{ab}$
(mmol/l)	LE	3·23	$0·10^{a}$	2·56*	$0·11^{b}$	2·78*	$0·11^{b}$	3·03	$0·09^{a}$
Non-esterfied fatty acids	HE	121	26^{ac}	283	36^{b}	154	18^{c}	109	15^{a}
(μmol/l)	LE	178	34^{ac}	375*	29^{b}	217	24^{c}	117	16^{a}
Ketone bodies	HE	545	32^{a}	722	53^{b}	701	38^{b}	635	27^{b}
(μmol/l)	LE	485	13^{a}	1493*	157^{b}	1176*	135^{b}	750	54^{c}
Protein	HE	75·0	1·3	81·1	1·4	84·6	$1·1^{b}$	84·0	$1·4^{b}$
(g/l)	LE	70·6*	$1·1^{a}$	75·0*	$1·2^{b}$	77·1*	$1·4^{b}$	76·9*	$1·1^{b}$
Albumin	HE	35·4	0·7	35·9	0·6	37·0	0·6	37·0	0·6
(g/l)	LE	33·9	$0·4^{a}$	33·8*	$0·5^{a}$	34·7*	$0·6^{ab}$	35·9*	$0·5^{b}$
Urea	HE	5·24	$0·26^{a}$	6·21	$0·43^{ab}$	6·84	$0·49^{b}$	5·74	$0·28^{ab}$
(mmol/l)	LE	4·03*	$0·24^{a}$	2·93*	$0·24^{b}$	2·54*	$0·18^{b}$	3·54*	$0·19^{a}$
Somatomedin C	HE	69·7	$8·5^{a}$	27·3	$4·0^{b}$	41·8	$8·0^{b}$	42·7	$8·7^{b}$
(μg/l)	LE	45·6*	$6·5^{a}$	13·7*	$1·6^{b}$	24·2	$4·4^{c}$	30·5	$5·2^{c}$
Somatotropin	HE	2·62	$0·46^{a}$	4·96	$0·64^{b}$	4·78	$0·73^{b}$	3·73	$0·50^{ab}$
(μg/l)	LE	2·98	$0·36^{a}$	6·91*	$0·77^{b}$	7·62*	$1·14^{b}$	6·74*	$0·97^{b}$
Immunoreactive insulin	HE	573	50^{a}	375	24^{b}	433	29^{b}	450	30^{b}
(ng/l)	LE	517	35^{a}	228*	9^{b}	297*	18^{c}	380	27^{d}
Triiodothyronine	HE	2·44	0·24	1·92	0·15	2·23	0·15	2·38	0·20
(nmol/l)	LE	2·17	$0·18^{a}$	1·58	$0·16^{b}$	1·97	$0·16^{ab}$	2·25	$0·14^{a}$
Thyroxine	HE	88·9	$4·3^{a}$	64·6	$2·3^{b}$	72·7	$2·8^{c}$	80·2	$3·1^{ac}$
(nmol/l)	LE	81·2	$5·0^{a}$	57·4*	$2·1^{b}$	75·4	$2·6^{a}$	86·4	$3·6^{a}$

		Experiment C							
				Weeks of lactation					
		Dry period		1 to 4		5 to 8		9 to 12	
		Mean	s.e.	Mean	s.e.	Mean	s.e.	Mean	s.e.
Glucose	HE	3·08	0·09[a]	2·62	0·05[b]	2·61	0·09[b]	2·84	0·04[c]
(mmol/l)	LE	3·03	0·05[a]	2·71	0·08[b]	2·68	0·06[b]	2·86	0·06[ab]
Non-esterfied fatty acids	HE	165	17[ac]	280	17[b]	193	16[a]	148	6[c]
(μmol/l)	LE	189	21[a]	244	24[a]	197	19[a]	135	9[b]
Ketone bodies	HE	760	81[a]	1191	138[b]	1757	187[b]	1271	175[b]
(μmol/l)	LE	849	96[a]	1469	159[b]	1924	268[b]	1460	132[b]
Protein	HE	70·6	2·2	69·9	0·9	71·9	1·0	71·6	0·9
(g/l)	LE	69·0	1·5	69·8	0·6	70·0	0·5	70·5	0·7
Albumin	HE	34·9	0·9[a]	36·9	0·3[a]	38·1	0·3[b]	37·9	0·3[b]
(g/l)	LE	35·3	0·3[a]	37·4	0·3[b]	37·3	0·3[b]	38·3	0·3[b]
Urea	HE	3·32	0·33[a]	4·46	0·16[b]	4·77	0·09[b]	3·37	0·11[a]
(mmol/l)	LE	4·27	0·29[a]	3·24*	0·11[b]	3·25*	0·13[b]	3·67	0·11[a]
Somatomedin C	HE	67·2	16·2[a]	22·2	2·7[b]	26·3	2·3[b]	30·7	2·9[b]
(μg/l)	LE	64·5	11·2[a]	19·2	1·5[b]	25·5	1·6[c]	32·5	2·0[d]
Somatotropin	HE	1·87	0·33[a]	4·46	0·35[b]	4·03	0·43[b]	3·34	0·31[b]
(μg/l)	LE	1·90	0·32[a]	4·98	0·27[b]	4·34	0·59[bc]	2·84*	0·28[ac]
Immunoreactive insulin	HE	681	144[a]	318	27[b]	497	44[a]	559	47[a]
(ng/l)	LE	737	152[a]	336	34[b]	527	58[a]	552	49[a]
Triiodothyronine	HE	2·22	0·09[a]	2·29	0·06[a]	2·75	0·04[b]	3·01	0·09[c]
(nmol/l)	LE	2·37	0·17[a]	2·41	0·10[ac]	2·81	0·13[bc]	2·95	0·06[b]
Thyroxine	HE	77·6	3·6[ac]	66·7	2·3[b]	74·2	1·4[a]	79·4	1·8[c]
(nmol/l)	LE	89·6	6·1[a]	66·3	1·2[b]	72·7	1·7[c]	80·8	1·5[a]

[a,b,c] Different superscripts indicate significant differences between dry period, week 1 to 4, week 5 to 8 or 9 to 12 of lactation ($P < 0·05$).

* Significant difference between groups LE and HE and LD and HD ($P < 0·05$).

DISCUSSION

In the study lasting over the entire lactation, energy and protein balances were always positive, because of a relatively low milk yield. In further experiments, in which high-yielding cows were used, energy and protein balances became markedly negative in the first months of lactation, even when given high amounts of concentrates. Only with the feeding of crystalline fat were calculated energy and protein balances positive after parturition.

FCM yield was either unaffected or only slightly reduced in cows consciously underfed with energy and protein. Marked energy deficiency was associated with decreased milk protein and in part with increased milk fat, marked protein deficiency with decreased milk protein. Feeding high amounts of crystalline fat as in this study caused lowered milk fat and protein concentration.

As shown previously (Blum *et al.*, 1985a; Kunz *et al.*, 1985; Mills, Beitz and Young, 1986), negative energy balances were typically characterized by low glucose and high NEFA and ketone body levels, suggesting insufficient gluconeogenesis, enhanced fat mobilization and ketogenesis. Negative protein balances were typically characterized by low urea concentrations, whereas an oversupply of protein when energy balances were negative was followed by elevated urea concentrations. Combined low energy and protein intake led to low circulating urea, partially low albumin and protein and high NEFA and ketone body concentrations (Oldham, Broster, Napper and Siviter, 1979; Oltner and Wiktorsson, 1983; Clement, 1988). On the other hand, feeding crystalline fat improved energy supply and was characterized by relatively high blood glucose, relatively low levels of NEFA and ketone bodies (Blum *et al.*, 1985a).

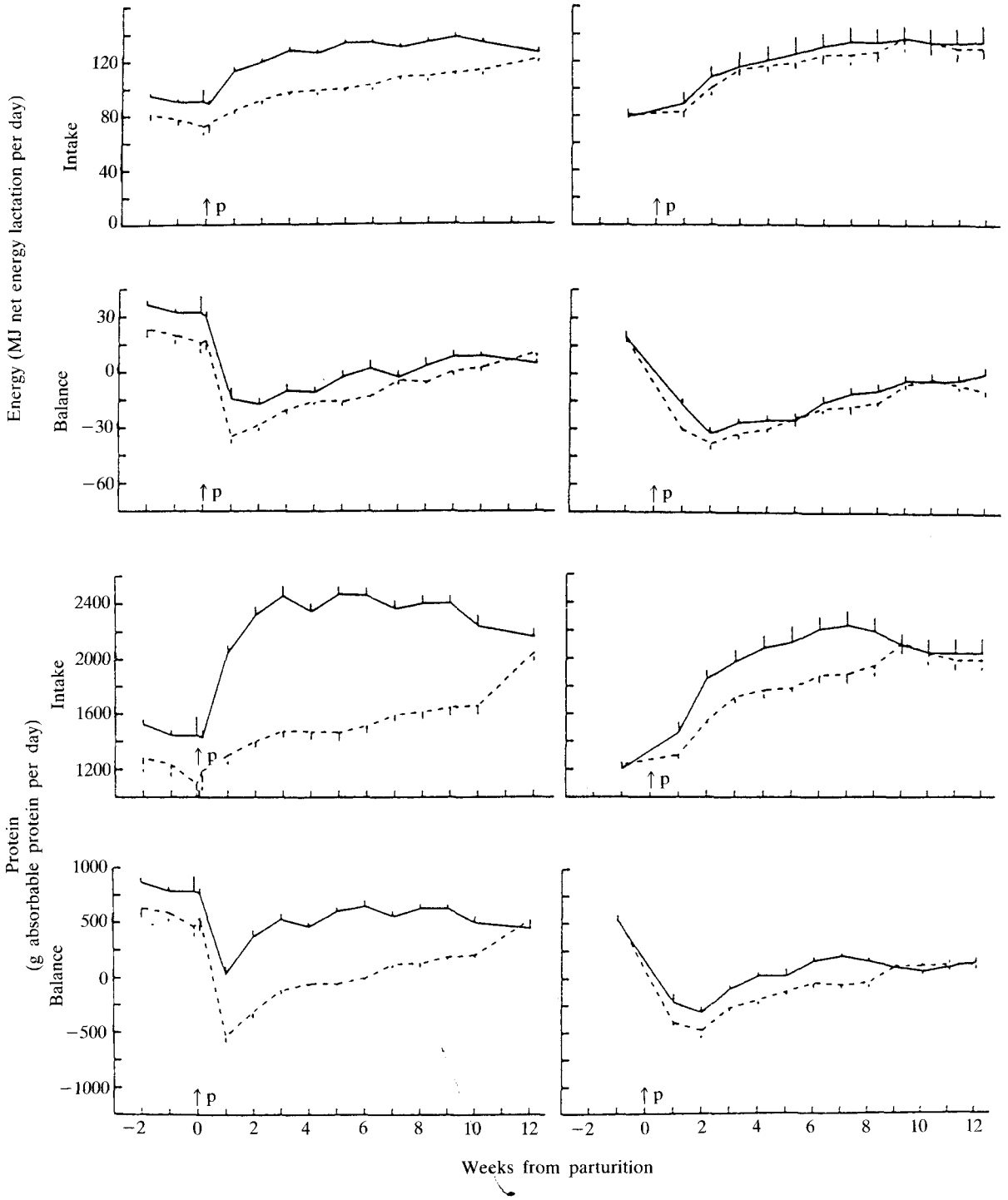

FIG. 2. Energy and protein intake and balances of experiments B and C (on left and right side, respectively). Each point represents the mean ± s.e. of a 7-day period. ———, groups HE and LD, respectively - - - -, groups LE and HD, respectively. P = parturition.

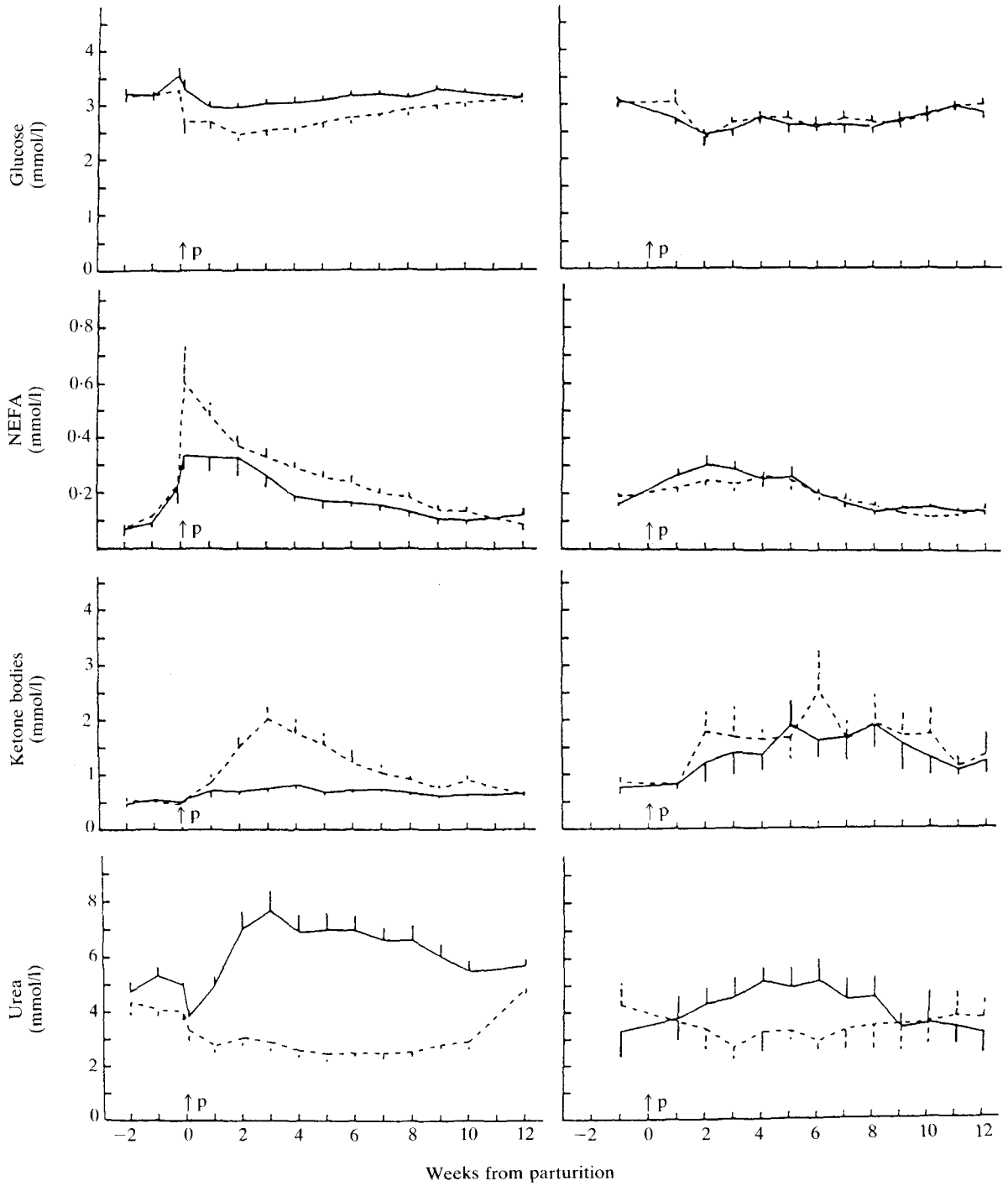

FIG. 3. Blood levels of glucose, non-esterified fatty acid (NEFA), ketone bodies and urea (experiments B and C). For further details see legend to Figure 2.

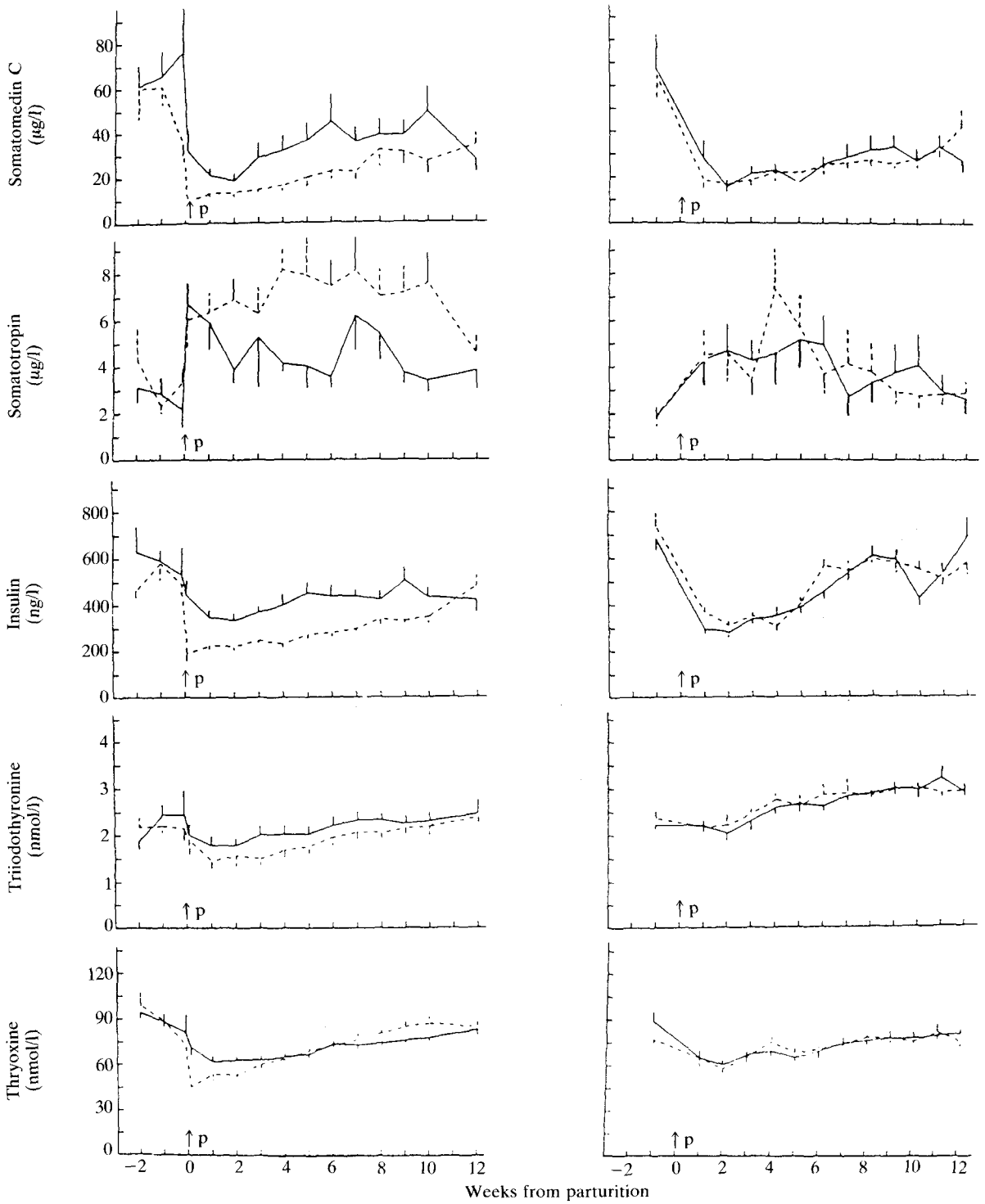

FIG. 4. Blood levels of somatomedin C, somatotropin, immunoreactive insulin, triiodothyronine and thyroxine (experiments B and C). For further details see legend to Figure 2.

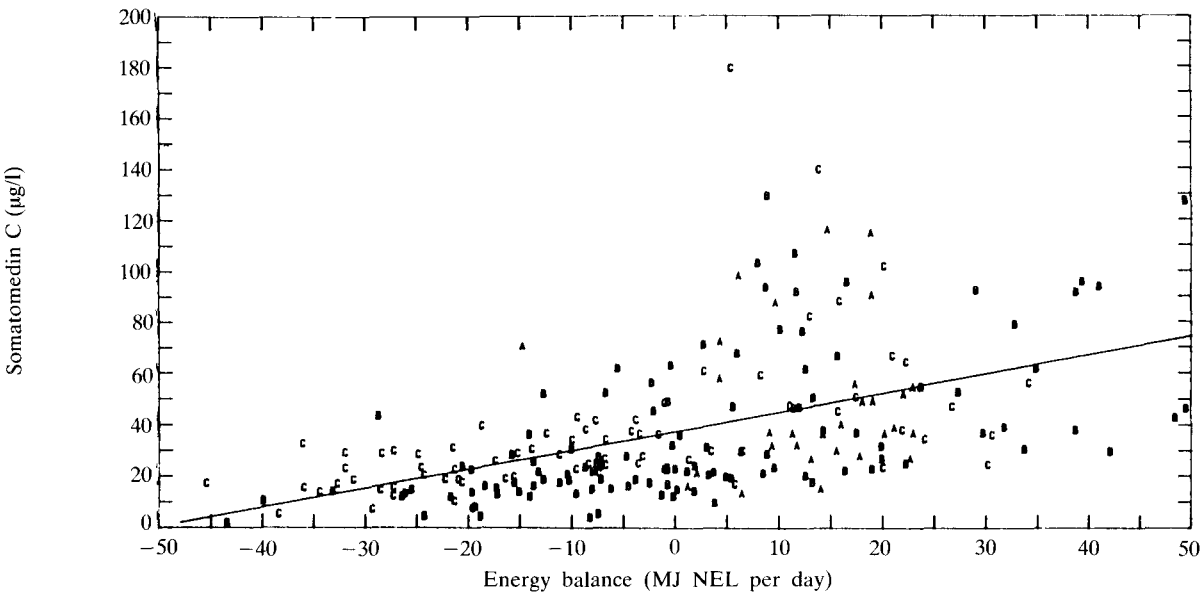

FIG. 5. Relationship between somatomedin C and energy balances from the dry period (2 weeks approx.) up to the 12th week of lactation. Each point represents the mean of 4 weeks for each animal from experiments A, B and C.

TABLE 3

Experiments B and C: correlation† of somatomedin C with various measurements

	Experiment		
	B	C	B and C
Net energy lactation intake	−0·09	−0·52	−0·25*
balance	0·64*	0·67*	0·61*
Absorbable protein intake	−0·04	−0·55*	−0·23*
balance	0·48*	−0·61*	0·47*
Fat-corrected milk yield	−0·47*	−0·68*	−0·51*
Glucose	0·38*	0·54*	0·38*
Non-esterified fatty acids	−0·49*	−0·09	−0·28*
Ketone bodies	−0·59*	−0·51*	−0·46*
Protein	0·04	−0·09	−0·07
Albumin	0·24*	−0·45*	0·02
Urea	0·28*	−0·15	0·15*
Somatotropin	−0·58*	−0·35*	−0·50*
Immunoreactive insulin	0·66*	0·27*	0·48*
Triiodothyronine	0·34*	−0·10	0·22*
Thryoxine	0·45*	0·39*	0·43*

† Correlations are calculated for each experiment over periods and animals. Degrees of freedom were: 74 for B, 118 for C and 194 for B and C.

Somatomedin C was very stable during the 24 h studied (Figure 6). In particular, SmC levels did not change in response to food intake. This is in accordance with studies in pigs (Sillence and Etherton, 1987). Thus, a single blood sample during the course of the day seems to be sufficient to characterize the SmC status. In contrast, Binnerts *et al.* (1982) and Falconer *et al.* (1980) reported diurnal variations of somatomedin-like activity which is, however, not identical with the specifically and radioimmunologically measured SmC in our investigation. The considerable stability of SmC levels over a 24-h period is likely to be the consequence of SmC binding to specific blood plasma proteins (Zapf, Hauri, Waldvogel and Froesch, 1986). One important consequence is a half-life of SmC in the circulation of several h (Froesch and Zapf, 1985). In addition, protein binding markedly reduces acute biological effects of SmC and is thought to be responsible for absence *in vivo* of most insulin-like effects found *in vitro*

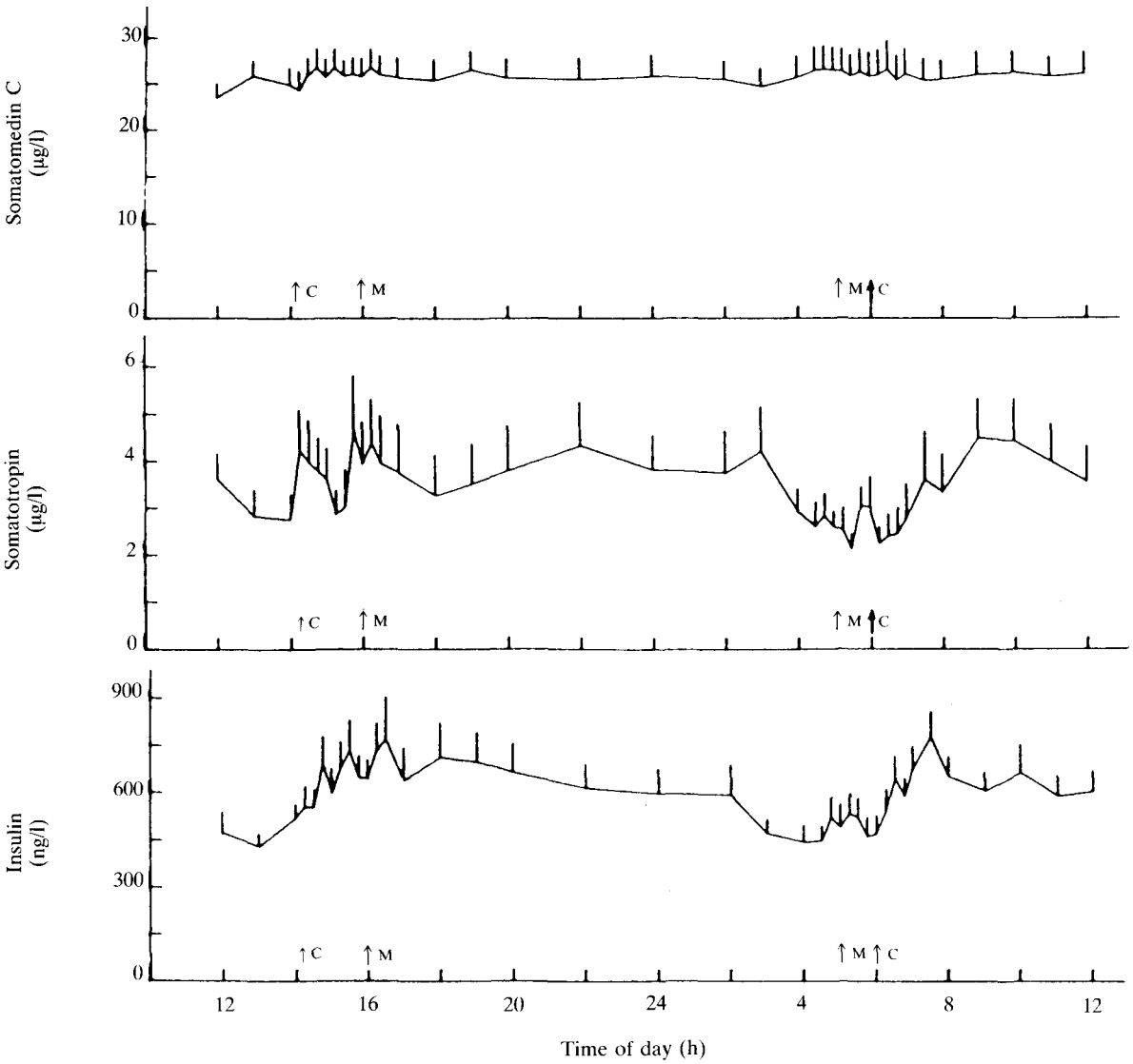

FIG. 6. Blood levels of SmC, STH and IRI during a 24-h period (mean ± s.e. of all 18 animals of experiment D). Animals received concentrate at 06.00 and at 14.30 h (C) and were milked at 05.15 h and at 16.00 h (M).

(Zapf *et al.*, 1986). IRI consistently increased (Bines, Hart and Morant, 1983) and STH appeared to rise only marginally after the intake of concentrates (irregularly also after milking), and thus are probably more important for rapid regulation of metabolism than SmC.

Our study clearly demonstrates a negative correlation between levels of specifically measured SmC and milk yield. Somatomedin-like activity behaved similarly (Binnerts *et al.*, 1982). In accordance, Falconer *et al.* (1980) found lower somatomedin-like activity in high, compared with low, yielding cows. Our data also indicate that the presence of a foetus, i.e. pregnancy *per se* (up to the 7th month of

TABLE 4

Body weight, dry matter (basic ration (BR) and concentrates (C)), net energy lactation (NEL) and crude protein (CP) intakes and absorbable protein (AP) intakes and balances, fat-corrected milk yields (FCM), milk composition, blood metabolites and hormones in lactating cows given different amounts of protein and energy (experiment D)†

	Group					
	LE		NE		CF	
	Mean	s.e.	Mean	s.e.	Mean	s.e.
Body weight (kg)	596	15	632	11	650	22
BR-intake (kg/day)	11·3	0·5[a]	11·6	0·5[ab]	13·0	0·6[b]
C intake (kg/day)	2·7	0·2[a]	6·6	0·0[b]	4·6	0·0[c]
NEL intake MJ/day)	88	4[a]	120	3[b]	128	4[b]
NEL balance (MJ/day)	−40	4[a]	−19	1[b]	1	4[c]
CP intake (g/day)	2188	85[a]	2630	60[b]	2648	62[b]
AP intake (g/day)	1489	59[a]	1895	44[b]	1994	45[b]
AP balance (g/day)	−288	65[a]	−41	39[b]	71	46[b]
FCM (kg/day)	30·4	1·5	31·0	1·3	29·3	2·2
Milk fat (g/l)	47·8	2·7[a]	37·7	1·1[b]	32·2	2·2[b]
Milk protein (g/l)	34·0	0·8[a]	32·0	1·1[ab]	30·3	0·3[b]
Milk lactose (g/l)	52·7	1·1	50·7	1·5	50·5	0·7
Glucose (mmol/l)	3·25	0·19	3·46	0·03	3·60	0·09
Non-esterified fatty acids (μmol/l)	257	63[ab]	145	6[a]	208	13[b]
Ketone bodies (μmol/l)	1872	363[a]	995	104[ab]	852	51[b]
Protein (g/l)	78·7	2·0	81·1	3·7	76·4	2·8
Albumin (g/l)	42·3	0·7	43·6	1·0	44·1	1·3
Urea (mmol/l)	5·42	0·47[ab]	3·90	0·39[a]	6·01	0·71[b]
Somatomedin C (μg/l)	17·1	3·1	23·9	2·2	27·3	4·8
Somatotropin (μg/l)	6·06	0·63[a]	2·67	0·56[b]	1·44	0·24[b]
Immunoreactive insulin (ng/l)	361	39[a]	713	98[b]	718	83[b]
Triiodothyronine (nmol/l)	1·68	0·12[a]	2·58	0·24[b]	2·28	0·15[b]
Thyroxine (nmol/l)	53·8	5·0	68·7	6·6	58·7	4·3

[a,b,c] Different superscripts indicate significant differences between LE, HE and CF ($P < 0·05$).
† Values are expressed as mean ± s.e. of 24 h (39 samples).

pregnancy), does not measurably modify SmC levels compared with non-pregnant cows. Somatomedins other than SmC, such as insulin-like growth factor II, are considered important for foetal development (Underwood and D'Ercole, 1984; Gluckman, 1986).

Energy and/or protein intake and consequent changes of blood levels of various hormones are known to be determining factors of SmC levels (Breier *et al.*, 1986; Elsasser *et al.*, 1986; Underwood *et al.*, 1986). The experiment lasting over the entire lactation demonstrated a positive correlation between SmC levels and energy and/or protein balance. Interestingly, such a relationship was observed even though balances were always positive in this experiment, because milk production was low.

The fall of SmC levels after parturition was more marked in cows consciously undersupplied with energy, whereas an additional protein deficiency did not further lower SmC levels. High energy intake in the form of crystalline fat led to increased SmC levels. Moreover, energy balances were significantly and positively correlated with SmC levels. Thus, energy supply seemed greatly and dominantly to influence circulating SmC, whereas protein supply appeared to be of rather secondary importance. In accordance, in heifers during fasting for 3 days, SmC levels rapidly decreased and

returned to the normal range within 5 days of refeeding (H. Ronge and J. Blum, unpublished observations). Nevertheless, it has been shown that protein intake in growing steers has decisive effects on SmC levels (Elsasser *et al.*, 1986) and we also found a positive correlation between SmC and protein balance. However, energy and protein balances were strongly correlated in our experiments and thus the effects of energy and protein intake and balance are difficult to separate. Microbial protein synthesis in the rumen is greatly dependent on energy supply and there are important interactions between intermediary energy and protein metabolism (Macrae and Lobley, 1984).

Concentrations of STH increased after parturition, as shown previously (Hart, Bines, Morant and Ridley, 1978; Kunz and Blum, 1985). The increase was more marked the greater the degree of energy deficiency and tended to be elevated in cows undersupplied with protein and/or nitrogen, whereas very low STH levels were found in cows given high amounts of energy in the form of crystalline fat, indicating that STH is inversely related to energy intake (Blum *et al.*, 1985a). This is in accordance with other studies in lactating and non-lactating cattle (Hart *et al.*, 1978; Blum, Schnyder, Kunz, Blom, Bickel and Schürch, 1985b) and in other species. STH inhibits lipogenesis and favours lipolysis, enhances gluconeogenesis, stimulates protein deposition and retention of certain minerals and causes insulin resistance (Hart and Johnnson, 1986). Thus, STH is involved in regulation of metabolic changes seen in high-yielding dairy cows particularly in early periods of lactation or during energy and protein deficiency. The stimulation of milk production by STH during established lactation is thought to be largely the consequence of enhanced availability of substrates (particularly glucose) to the mammary gland for milk synthesis, in part indirectly due to stimulation of blood flow through the udder, whereas a direct effect on mammary tissue has not yet been demonstrated (Hart and Johnsson, 1986; Karg and Mayer, 1987). How STH enhances the synthetic capacity of individual alveolar cells, which is expected to be a prerequisite for enhanced milk formation, is unclear. However, specific SmC binding to bovine mammary tissue has been found (Baumrucker, 1986a and b). Thus, SmC could be important for mammary gland growth and alveolar cell regeneration, especially during the dry period. This is partially supported by much higher SmC levels during the 1st day *post partum* in colostrum than in blood (H. Ronge and J. W. Blum, unpublished observations). It remains to be shown whether SmC is produced locally in the mammary gland, is regulated and has effects distinct from SmC produced in the liver, i.e. 'systemic' SmC. Because we found low circulating SmC levels in early lactation, blood SmC effects on mammary tissue may be negligible during this time period.

Somatotropin is well known to enhance the production of SmC in liver, although a direct effect on SmC production in bovine liver cells has to our knowledge not yet been demonstrated. Furthermore, SmC levels increased during administration of STH in cattle (Davis, Gluckman, Hart and Henderson, 1987). Therefore, low levels of SmC in the presence of high concentrations of STH, i.e. a negative correlation between SmC and STH, as found in this study particularly in the first period of lactation, was unexpected. Several causes could be responsible for this paradoxical situation. Because SmC is markedly decreased in fasted animals, including cattle (H. Ronge and J. W. Blum, unpublished observations), the low energy (and protein) supply and hence decreased availability of energy yielding substrates and amino acids, in early lactation could be a primary link. Furthermore, SmC producing liver cells may be less sensitive or responsive to STH during energy and protein deficiency, as found in the rat (Maes, Underwood and Ketelsleger, 1984). Thus, SmC responses to exogenous STH are greatly decreased in heifers after 3 days of fasting and in high-yielding lactating cows in early lactation, as compared with the dry period (H. Ronge and J. W. Blum, unpublished observations). Similarly, SmC responses to STH were reduced in humans during starvation (Merimee, Zapf and Froesch, 1982). Low circulating insulin and thyroid

hormone levels, as found in this study, may contribute to a smaller effect of STH on SmC production because the presence of these hormones is known to be essential for STH to induce its effect in liver tissue and other hormones, such as cortisol, may directly decrease SmC production (Spencer, 1985). In addition, disturbed functioning of liver cells, including SmC producing cells, as a consequence of energy deficiency (Mills *et al.*, 1986), may also cause reduced SmC synthesis.

High circulating STH and relatively low blood levels of SmC and insulin in early lactation should change metabolism in a direction favouring high milk yield by mobilization and enhanced oxidation of fat, but reduced oxidation of glucose, which is preferentially needed for lactose synthesis. On the other hand, high SmC and insulin together with normal STH concentrations during the recovery period of lactation and during the dry period can be expected to stimulate deposition of body tissues, such as fat, muscle and bone. The typical changes of T_4 and T_3 seen also in this study during the lactation cycle seems to be primarily associated with shifts in energy metabolism, especially heat production (Blum, Kunz, Leuenberger, Gautschi and Keller, 1983; Blum *et al.*, 1985a; Kunz and Blum, 1985; Kunz *et al.*, 1985). They are known to modify the sensitivity of various tissues such as the liver to STH (Spencer, 1985) or other hormones. We have also proposed that low T_4 and T_3 concentrations in early lactation could help to conserve muscle mass (Blum *et al.*, 1985a). This is thought to be an important effect also of STH, but only when insulin circulates in normal amounts (Bines, Hart and Morant, 1980).

In conclusion, the changes of SmC seen in these experiments seem to be primarily regulated by energy balance and secondly by protein balance. The reduced production of SmC, while STH is elevated, might additionally be influenced by low insulin, T_3 and T_4, as a consequence of the negative energy balances. Thus, rather than a single endocrine factor, only a well orchestrated interplay of various endocrine systems will enable cows finally to produce high amounts of milk.

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