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SERUM CONCENTRATIONS OF INSULIN-LIKE GROWTH FACTORS AND THYROID HORMONES IN HEALTHY AND KETOTIC DAIRY COWS DURING THE PUERPERIUM

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The purpose of this investigation was to compare peripheral circulating concentrations of the insulin-like growth factors (IGF) and thyroid hormones (T3 and T4) during the periparturient period in dairy cows in different situations. In Study 1 from an average of about 4 days prepartum to 4 days postpartum the decreases in serum T3, T4 and IGF-I were more marked in a group of cows (n = 24) with mean body condition score (BCS) 4.2 than in a group (n = 24) with mean BCS 3.2. Serum IGF-II increased, thereby maintaining a relatively constant molar sum for the two growth factors. In a second study daily samples taken in March from twelve healthy cows over the same interval showed similar alterations with a statistically significant minimum for serum IGF-II on the day of calving. Opposite time-related trends were observed for T3, T4 and IGF-I in this group in comparison with a later group of healthy cows (n = 12) which received an equivalent diet containing fresh green lucerne instead of ensiled lucerne hay. Levels postpartum of all four hormones were low in cows (n = 12) exhibiting ketonuria. While the closest correlations were found between serum T3 and T4 levels, there were significant independent relationships between the levels of one or other thyroid hormone and serum IGF-I concentration. The characteristic interrelationships between the four hormones were maintained in both studies indicating that mechanisms controlling their synthesis and degradation are interdependent and preserved under some conditions of nutritional excess and deficiency.
Key words: body condition, insulin-like growth factors, ketosis periparturient cows, thyroid hormones

INTRODUCTION

The continuous decline in total circulating IGF-I concentrations from late gestation into early lactation in both beef and dairy heifers and cows has been well documented (Hadsell *et al.*, 1993; Hoshino *et al.*, 1991; Hossner *et al.*, 1997a;

Schams *et al.*, 1991; Simmons *et al.*, 1994; Skaar *et al.*, 1991; Vega *et al.*, 1991) and related to the marked deficiency in nutrient supply at this period, particularly concerning metabolizable protein balance (Bell *et al.*, 2000; Nielsen and Riis, 1993; Ronge *et al.*, 1988). Mechanisms for the decrease appear to include down regulation of a liver specific promoter for growth hormone (GH) receptor messenger ribonucleic acid (mRNA), as well as decreases in both class 1 and class 2 IGF-I mRNA ((Kobayashi *et al.*, 1999). In contrast to IGF-I, the few data collected for circulating IGF-II in periparturient cows indicated constant concentrations (Hossner *et al.*, 1997a; Skaar *et al.*, 1991; Vega *et al.*, 1991), although marked changes in response to different treatments have been observed in beef heifers and sheep (Hill *et al.*, 1999; Oldham *et al.*, 1999).

Both peptides act to promote growth, development and differentiation, the activity of IGF-I being recognized as several fold more potent than that of IGF-II (Hossner *et al.*, 1997a). Besides these ligands, the IGF system includes high affinity receptors and binding proteins (IGFBPs), six of which associate with the IGFs with high affinity, modifying their distribution and activity. Changes in the relative amounts of circulating IGFBPs occurred during gestation and lactation in cattle (Baumrucker and Erondu, 2000; Simmons *et al.*, 1994; Skaar *et al.*, 1991; Vega *et al.*, 1991; Vleurick *et al.*, 1999). For example, IGFBP-2 increased dramatically around the onset of lactation and then decreased, whereas IGFBP-3, which can form stable higher molecular weight ternary complexes retained within the circulatory system, tended to follow a delayed lactation curve.

The positive correlation between circulating thyroid hormone concentrations and energy balance in cows is well known (Kunz and Blum 1985; Pethes *et al.*, 1985). It is probable that alterations in blood levels of thyroxine (T4) primarily reflect changes in thyroid secretion rate, as found for goats (Riis and Madsen, 1985), while peripheral triiodothyronine (T3) concentrations are influenced mainly by extra-thyroidal deiodinase (D) activity. In the cow the highly efficient type II 5'D predominates in the mammary gland, enabling T3 production in support of lactation to proceed at the expense of other tissues, such as the liver, where the type I 5'D prevails (Slebodzinski *et al.*, 1999). Moreover, hypothyroidism has been shown to affect the IGF system, leading to altered serum levels of both ligands and binding proteins in man, rodents and pigs (Miell *et al.*, 1993; Näntö-Salonen *et al.*, 1993; Nikolić *et al.*, 1998a, Sugisaki *et al.*, 1993). Inadequate thyroid hormone status and/or the appearance of ketosis appeared to have independent effects on serum IGF-I concentrations in puerperal dairy cows ((Nikolić *et al.*, 1997).

In the present investigation the effects of different prepartal body condition, as well as the influence of conditions possibly leading to ketosis, on peripheral IGF and thyroid hormone concentrations were examined in multiparous dairy cows kept under the usual farm conditions. Peripheral serum concentrations reflect the balance between synthesis and degradation which are affected by multiple factors. The data obtained were analysed statistically in order to detect possible interrelationships in the metabolic control of the levels of these four hormones and any changes associated with excessive fatness prepartum or spontaneous ketosis postpartum.

MATERIALS AND METHODS

Study 1

A total of 48 Holstein-Friesian cows was divided into two equal groups (C and F) on the basis of body condition score (Edmondson *et al.*, 1989) at about 10 days before calving in early spring 1997. The cows were kept tethered in the maternity parlour of a large farm and received a mixed diet containing dehydrated lucerne meal, lucerne hay, maize silage, ensiled maize grain, wheat meal, sunflower oil meal, vitamins and minerals, contributing 18.2, 21.9, 24.3, 8.4, 18.7, 7.5 and 1.0% of the dry matter (DM) intake (12.35 kg per day) respectively. The mean (SD) body condition score (BCS) of the control group was 3.2 (0.5), which was significantly less (P .001) than 4.2 (0.6) measured in the fatter animals, while their previous 305 day milk yields were 6655 (1430) l and 7566 (1366) l respectively. The cows were about to enter their second to eighth lactation 3.1 (1.1) for Group C; 3.7 (1.6) for Group F. Blood samples were obtained by jugular puncture from each cow once during the last week before calving and once during the first week postpartum around 1100 hours in the morning about 4-6 hours after milking and feeding. No sign of clinical or subclinical ketosis was detected in any cow (inappetance, ruminal atony etc).

Study 2

Daily postprandial blood samples were taken as described above from a group of twelve Holstein-Friesian cows kept in the same manner on the same farm as in Study 1 from 4 days before calving to 4 days postpartum in March 1998 (Group P, Study 2a). A similar complete ration was offered, providing 12.35 kg DM and 1605 g crude protein but containing barley straw and ensiled lucerne hay instead of the first two ingredients used in Study 1. The diet was enriched postpartum by replacing the barley straw with soya grits and brewers' grains (14 kg DM and 2657 g crude protein per day). The cows had given a previous average milk yield of 7747 (1006) l and were entering their second to fourth lactation 3.25 (0.75). Except for one cow which had transient positive results (++) for ketonuria on days 2 and 3 postpartum, all the cows remained healthy throughout the puerperium in the maternity parlour. Since spontaneous primary ketosis was a problem at this time, blood samples were also obtained on days 1 to 4 postpartum from cows (Group K; n = 12) with persistent ketonuria (Rothera test +++ or ++) and typical clinical symptoms (loss of appetite, rumen atony, decreased milk production). Two months later in May, when ketosis was rare, blood samples were obtained from a second group of twelve healthy cows (Group H). The diet at that time contained fresh green lucerne in place of the ensiled lucerne hay. The previous milk yields were 8021 (732) and 7473 (1391) l and mean lactation number was 3.4 (0.9) and 2.7 (0.8) for Groups K and H respectively.

Analyses

After clotting spontaneously at room temperature, blood samples were centrifuged and the serum decanted and preserved at 18 ^{0}C for analysis. Total concentrations of IGF-I and IGF-II were determined in neutralised acid-ethanol extracts of serum using classical competitive binding systems ((Nikolić *et al.*, 1996, 1998b) as modified for bovine samples (Nikolić *et al.*, 2001). Briefly, the IGF-I

radioimmunoassay (RIA) was performed using human (h) IGF-I (purity = 98%; ICN Biomedicals Inc., Aurora, USA) labelled with ^{125}I as the tracer by the chloramine-T method and polyclonal rabbit antibodies to h-IGF-I (Biogenesis, Poole, UK) as the reagent. Since the aminoacid composition is the same as bovine IGF-I, h-IGF-I (0.063 - 6.25 ng/tube) was used as the working standard in the presence of h-IGF-II (4 ng/tube). Cross reactivity with IGF-II at 50% inhibition of tracer binding was 0.054%. Mean (SEM) recovery of IGF-I from representative spiked samples (n = 16) was 93.4 (5.3)% of the expected increment. Reproducibility was checked by including reference IGF-I (WHO 87/518) in each test. The mean intraassay coefficients of variation (CV) for duplicate samples were routinely from 3 to 6%. Interassay CVs were below 12%.

For the IGF-II RIA, a mouse monoclonal anti-rat IGF-II (Biogenesis, Poole, UK) was used as the reagent (cross reactivity with IGF-I was 0.4% at 50% inhibition of tracer binding) and h-IGF-II (ICN Biomedicals Inc., Aurora, USA) labelled with ^{125}I as the tracer. Bovine IGF-II partially purified by molecular sieve chromatography in 1M acetic acid was used as the working standard (Nikolić *et al.*, 2001). Reproducibility was monitored by including fresh extracts of the same human serum pool in each test (intraassay CVs 3 - 6%; interassay CVs 13%). Mean (SEM) recovery of h-IGF-II was 91.2 (4.0)% of the expected increment for representative sera (n = 20).

Commercial RIA kits were used to determine T3 and T4 in accordance with the instructions (INEP, Zemun, Yugoslavia). The mean intraassay CVs of duplicate samples were 4.1 and 3.5% respectively. Interassay CVs were below 10%. Among other analyses, blood glucose, serum insulin, NEFA were also determined by standard methods but the results will be given in detail elsewhere.

Statistical analysis.

The results were subjected to analysis of variance (ANOVA) using two-way (cow, time) or split-plot factorial (treatment, time, repetition) models with appropriate checks for heterogeneity of variance. The statistical significance of differences between group means was estimated by the least significant difference (LSD) test. Associations between different variables were sought by correlation and multiple regression analysis.

RESULTS

Study 1

The results in Table 1 show marked differences between the two groups of cows for serum concentrations of IGF-I, T3 and T4 prepartum but not for values obtained postpartum. Decreases in mean values over the periparturient period were apparent for T3, T4 and IGF-I in both groups of cows but the change in IGF-I concentration was not statistically significant for the cows in normal body condition. The decline in thyroid hormone levels was steeper in the group of fat than in control cows. Serum IGF-II concentrations tended to increase but the change was significant only for the group of fat cows. The molar sum of the two growth factors was remarkably constant suggesting that overall serum IGF binding capacity remained stable. An overall impression of the changes with time for both groups of cows is presented in Figures 1 and 2.

Table 1. Mean levels of thyroid hormones and IGFs (nmol/l) in serum from Holstein -Friesian cows of different body condition before and after calving (study 1)

Group	Time PP	IGF-I	IGF-II	Molar IGF sum	T3	T4
C - control;	-3.6^{b}	6.3^{b}	26.4^{ab}	32.7^{a}	2.40^{b}	46.5^{b}
n = 24	4.3^{a}	5.1^{bc}	30.0^{a}	35.1^{a}	1.74^{c}	35.8^{c}
F - fat;	-4.9^{c}	9.3^{a}	22.8^{b}	32.1^{a}	3.38^{a}	67.8^{a}
n = 24	4.1^{a}	4.0^{c}	29.4^{a}	33.4^{a}	1.72^{c}	41.8^{bc}
SEM	0.4	0.7	1.8	2.0	0.15	3.0
F (state)	2.7	0.87	1.36	0.37	7.28	8.95
P-value	NS	NS	NS	NS	0.0097	0.0044
F (time)	449	21.1	8.24	0.88	60.0	36.5
P-value	<0.0001	<0.0001	0.0062	NS	<0.0001	<0.0001
F (inter.)	1.6	8.2	0.69	0.08	11.18	6.27
P-value	NS	0.0063	NS	NS	0.0017	0.016

^{abc} Means in the same column not sharing superscripts are significantly different ($P < 0.05$)

PP - postpartum (days)

While mean sampling times postpartum corresponded closely for both groups, the samples were obtained before calving somewhat earlier (-4.9 days) from the group of fat cows than from the control group (-3.6 days). This was due to the difficulty in predicting parturition dates accurately. In order to check if the difference in sampling time affected the significance of the differences between the groups, the results for two or more cows taken on the same day were randomly averaged to provide the same number of data for each day in each group e.g. for day - 4 the results for cows 2 and 13 and for cows 19 and 22 in the control group were averaged to provide two sets of data equivalent to data for cows 9 and 13 in Group F. In this way sixteen individual sets of data with identical time distributions were obtained for each group of 24 cows. One-way-ANOVA showed that the differences in thyroid hormone concentration between the two groups remained statistically significant ($F = 14.6$; $P<0.001$ for T3 and $F = 4.36$; $P= 0.045$ for T4), indicating a possible relationship between serum levels of these hormones and body condition prepartum. For IGF-I the difference between the groups was no longer statistically significant.

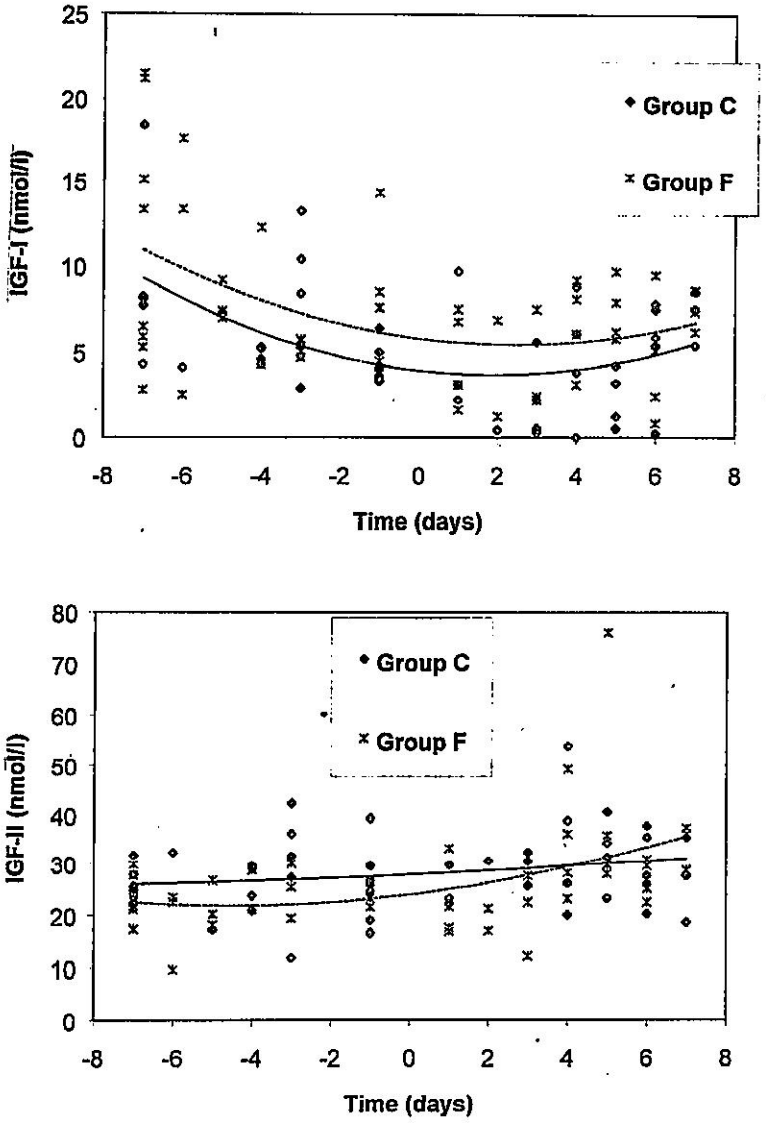

Figure 1. Serum IGF concentrations during the fortnight spanning parturition in multiparous Holstein-Friesian cows with normal (Group C) or high (Group F) body condition score (n = 48 x 2). Group C - continuous trendline. Group F - broken trendline.

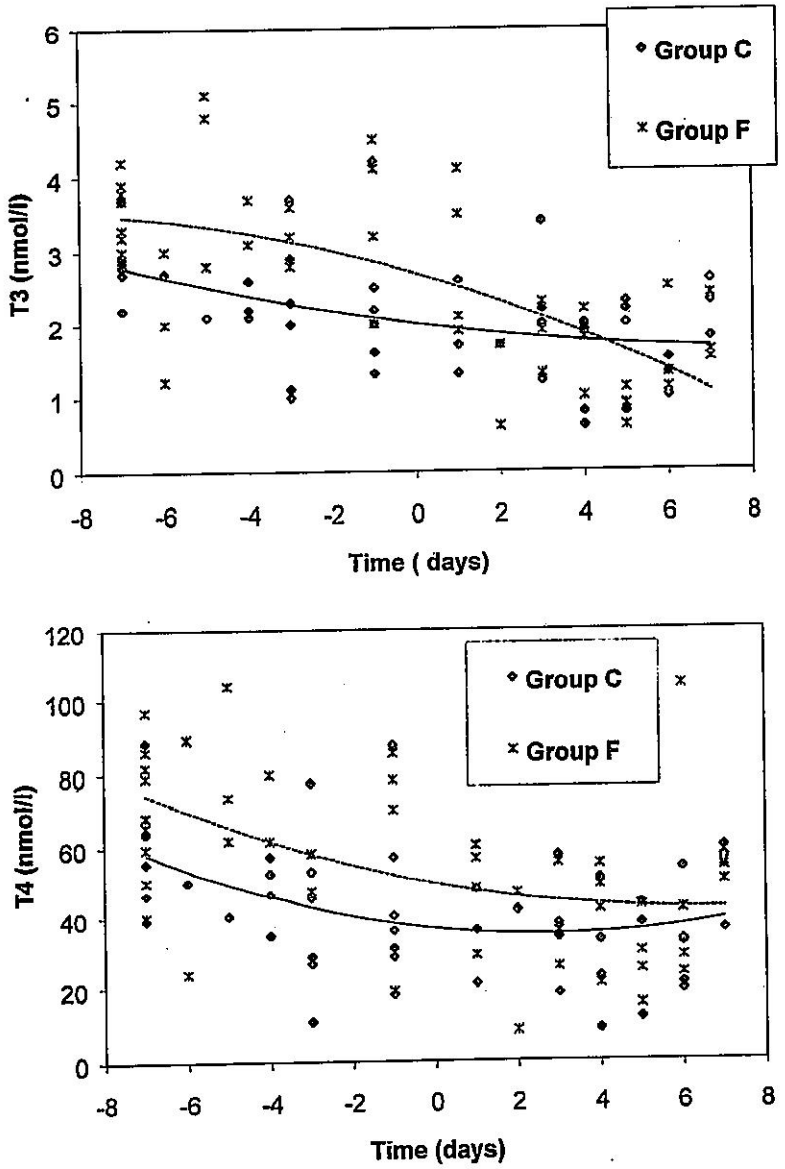

Figure 2. Serum thyroid hormone concentrations during the fortnight spanning parturition in multiparous Holstein-Friesian cows with normal (Group C) or high (Group F) body condition score (n = 48 x 2). Group C - continuous trendline. Group F - broken trendline

Study 2

While statistically significant differences between individual animals in Study 2a occurred for each hormone examined (Table 2), the changes with time were predominant for the thyroid hormones as indicated by the higher F-value. Non-additivity for the effects of each variable was apparent only for the IGF-I and combined IGF data. This was successfully eliminated by square root transformation without affecting the significance of the main effects. Changes up to the day before calving were statistically significant only for T3 which showed an

Table 2. Concentrations of the IGFs and thyroid hormones in serum (nmol/l) from twelve dairy cows during the peripartal period (study 2a)

Time PP	IGF-I	IGF-II	Molar IGF sum	T3	T4
-4	10.0^{a}	18.3^{abc}	28.3^{ab}	1.76^{b}	44.4^{ab}
-3	10.4^{a}	18.4^{abc}	28.8^{a}	1.87^{ab}	51.3^{a}
-2	10.2^{a}	19.7^{a}	29.9^{a}	1.84^{ab}	51.1^{a}
-1	9.8^{ab}	18.4^{ab}	28.2^{ab}	2.16^{a}	50.1^{a}
0	8.4^{bc}	16.0^{c}	24.4^{cd}	1.41^{c}	36.8^{c}
1	7.2^{cd}	16.5^{bc}	23.7^{cd}	1.32^{c}	35.4^{c}
2	6.9^{cd}	19.0^{a}	25.9^{bc}	1.31^{c}	38.1^{bc}
3	6.5^{cd}	18.3^{ab}	24.9^{cd}	1.18^{c}	33.1^{cd}
4	6.3^{cd}	16.5^{bc}	22.8^{d}	1.15^{c}	28.0^{d}
F (cow)	11.17	4.97	9.87	2.69	7.60
P-value	<0.0001	<0.0001	<0.0001	0.0049	<0.0001
F (time)	10.24	2.38	7.97	9.57	11.30
P-value	<0.0001	0.0229	<0.0001	<0.0001	<0.0001
F non-add.	5.73*	1.29	4.08*	2.30	0.97
P-value	0.01	NS	0.05	NS	NS

^{abcd} Means in the same column not sharing superscripts are significantly different ($P < 0.05$).

* Square root transformation abolished non-additivity without affecting main F-values

PP - postpartum (days)

increase. Marked decreases were then noted for all four hormones. Mean IGF-II concentration returned to the level observed prepartum within 2 days, while IGF-I and T4 declined further but T3 remained constant.

Table 3. Differences in postpartal serum IGF and thyroid hormone concentrations (nmol/l) between groups (n = 12) of healthy and ketotic cows (study 2b)

Group	Time PP	IGF-I	IGF-II	Molar IGF sum	T3	T4
P healthy	1	7.2^{ab}	16.5^{de}	23.7^{c}	1.32^{b}	35.4^{ab}
	2	6.9^{b}	19.0^{cde}	25.9^{c}	1.31^{b}	38.1^{a}
	3	6.5^{b}	18.3^{cde}	24.9^{c}	1.18^{bc}	33.1^{abcd}
	4	6.3^{b}	16.5^{e}	22.8^{c}	1.15^{bc}	28.0^{cde}
K ketonuria	1	4.3^{d}	22.3^{c}	26.6^{c}	0.79^{d}	25.6^{e}
	2	4.5^{d}	19.2^{cde}	23.7^{c}	0.82^{d}	29.5^{bcde}
	3	4.7^{cd}	17.7^{de}	22.5^{c}	0.82^{d}	25.7^{e}
	4	4.4^{d}	20.8^{cd}	25.2^{c}	0.92^{cd}	27.4^{de}
H healthy	1	5.9^{bc}	36.2^{a}	42.2^{a}	1.23^{b}	27.8^{de}
	2	6.6^{b}	29.3^{b}	35.9^{b}	1.31^{b}	34.0^{abc}
	3	8.3^{a}	33.0^{ab}	41.3^{a}	1.42^{ab}	34.8^{ab}
	4	6.9^{b}	32.5^{ab}	39.4^{ab}	1.71^{a}	38.9^{a}
SEM		0.49	1.51	1.64	1.51	2.17
F (group)		6.64	26.65	27.09	26.65	2.36
P-value		0.0038	<0.0001	<0.0001	<0.0001	NS
F (time)		1.33	1.59	1.06	1.59	1.99
P-value		NS	NS	NS	NS	NS
F (interaction)		1.74	2.20	1.85	1.92	3.55
P-value		NS	0.049	0.037	0.084	0.0031

^{abcde} Means in the same column not sharing superscripts are significantly different ($P < 0.05$)

PP - postpartum (days)

Since cases of ketosis were frequent in the maternity parlour at this time, a comparison of the concentrations of serum IGFs and thyroid hormones was made between the results found postpartum for this group and similar groups of cows, the first contemporary and overtly ketotic and the second a later set of healthy cows receiving a slightly different diet (Study 2b; Table 3). Thyroid hormone

Table 4. Correlation coefficients (r) between IGF and thyroid hormone levels for different groups of serum samples from periparturient cows

Study	Group	n	Variable	T4	IGF-I	IGF-II
1.	All (48 cows)	96	T3	0.776	0.528	NS
			T4	-	0.651	NS
			IGF-I	-	-	NS
	Group C (24 cows)	48	T3	0.861	0.504	NS
			T4	-	0.530	0.318
			IGF-I	-	-	NS
	Group F (24 cows)	48	T3	0.715	0.530	-0.363
			T4	-	0.721	NS
			IGF-I	-	-	NS
	Prepartum (48 cows)	48	T3	0.743	0.360	NS
			T4	-	0.641	0.307
			IGF-I	-	-	NS
	Postpartum (48 cows)	48	T3	0.687	0.525	NS
			T4	-	0.494	NS
			IGF-I	-	-	NS
2.	Group P (12 cows)	108	T3	0.604	0.379	NS
			T4	-	0.469	NS
			IGF-I	-	-	NS
	Postpartum (36 cows)	143	T3	0.672	0.392	0.318
			T4	-	0.208	0.230
			IGF-I	-	-	NS

NS - Not statistically significant ($P > 0.05$)

concentrations increased in Group H, decreased in Group P and remained low in Group K during the first four days after delivery. Mean T3 and IGF-I concentrations in Group K were lower than those in each of the other groups at almost every time interval. On the other hand, mean serum IGF-II concentrations were higher in Group H than in both groups of samples taken in March. Significant decreases in IGF-II concentration were noted between days 1 and 2 for Group H and between days 1 and 3 for Group K. The molar sum of the two growth factors was higher than in Study 1 in the samples taken in May from cows receiving fresh green lucerne but lower in both groups receiving ensiled lucerne hay. Both these samples and those in Study 1 were taken in March.

Mean blood glucose concentration was 2.36, 1.96 and 3.10 (SEM 0.08) mmol/l; serum non-esterified fatty acids (NEFA) 0.42, 1.27 and 0.87 (0.04) mmol/l; and serum insulin 10.2, 12.8 and 38.1 (1.6) mIU/l for groups P, K and H respectively.

Correlation analysis

In general, the closest associations were between the two thyroid hormones (Table 4). Serum IGF-I concentrations were correlated with each, more strongly with T4 prepartum and with T3 postpartum. Statistically significant positive correlations between T4 and IGF-II levels were also present but they were usually weaker than those for IGF-I in the first study but stronger in the second where results for the day of calving were included. Multiregression analysis using T3 or T4 as the dependent variable showed that, besides their close mutual association, the relations with serum IGF-I concentrations were often independent (Table 5). Namely, overall, in the group of fat cows prepartum and in Study 2a, a considerable portion of the variance in T4 concentrations was independently associated with T3 and IGF-I concentrations. Postpartum it was T3 which showed independent association with T4 and IGF-I levels in both studies.

Table 5. Multiregression analysis with T3 or T4 as the dependent variable for groups where two independent associations are active

Study	Group*	DV	Independent variable						R^2
				t	P		t	P	
1.	All	T4	T3	8.72	<0.001	IGF-I	4.87	<0.001	0.683
	Group F	T4	T3	4.62	<0.001	IGF-I	4.74	<0.001	0.674
	Prepartum	T4	T3	6.86	<0.001	IGF-I	5.02	<0.001	0.712
	Postpartum	T3	T4	4.74	<0.001	IGF-I	2.07	0.044	0.517
2.	Group P	T4	T3	6.27	<0.001	IGF-I	3.53	0.001	0.432
	Postpartum	T3	T4	10.32	<0.001	IGF-I	4.42	<0.001	0.518

DV - Dependent variable

* Groups as described in Table 4

DISCUSSION

The changes observed in circulating IGF-I and thyroid hormone concentrations in our cows confirm those found by other authors (Hadsell *et al.*, 1993; Hoshino *et al.*, 1991; Hossner *et al.*, 1997a; Kunz and Blum 1985; Pethes *et al.*, 1985; Schams *et al.*, 1991; Simmons *et al.*, 1994; Skaar *et al.*, 1991; Vega *et al.*, 1991). Moreover, concentrations of IGF-II were also within the range reported in the available literature (Hill *et al.*, 1999; Hossner *et al.*, 1997a; Skaar *et al.*, 1991; Vega *et al.*, 1991). While the results from Study 1, in which only two samples, one pre- and one postpartum, were taken from a large number (n =48) of cows, failed to show parturition related changes in IGF-II concentrations, longitudinal examinations with multiple samples from individual animals (Study 2) showed definite minima in circulating IGF-II levels on the day of calving. This may reflect exit of IGFs from the circulation into colostrum which contains 50-185 nmol IGF per litre (Nikolić and Masnikosa 1998; Skaar *et al.*, 1991; Vega *et al.*, 1991). It may be calculated that about 200 nmol of total IGF appears in 2 l of the first colostrum which, assuming the plasma volume is 26 l in a 650 kg cow, is sufficient to decrease the plasma concentration by about 8 nmol/l, if all IGF is derived from the peripheral circulation. This corresponds with the decline observed here, supporting the theory that most colostral IGF is derived from plasma (Hadsell *et al.*, 1993; Prosser *et al.*, 1991; Schams *et al.*, 1991; Vega *et al.*, 1991). Total peripheral IGF concentrations recover quickly in healthy cows with IGF-II and IGFBP-2 preceding IGF-I and IGFBP-3.

Considerable apparently time-related fluctuations in IGF-II levels (around 27 to 15 nmol/l) over the gestation period were evident in six beef heifers, although no regression equation could be calculated (Hossner *et al.*, 1997a). The mean values (SEM) reported by Skaar and coworkers (1991) showed a decline from 26.0 (2.4) 4 days before parturition to 21.8 (0.5) 1 day before calving followed by an immediate increase to 28.6 (2.4) 1.5 days after calving but were measured in three cows only. Presumably the lack of statistical significance led to their conclusion that serum IGF-II concentrations were constant. Vega and coworkers (1991) showed weekly data for just four cows indicating divergence of serum IGF-I and IGF-II levels from 4 days prepartum to 3 days postpartum (mean IGF-II approximately 20 and 23 nmol/l respectively). Thus, none of the data presented by these authors are incompatible with the time-related changes in serum IGF-II found in the current investigation. When cows in midlactation were fasted, the decline in plasma IGF-I concentration from about 30 to 15 nmol/l commenced immediately and was continuous over 48 hours ((McGuire *et al.*, 1995). The drop in serum IGF-II levels from about 40 to 30 nmol/l occurred after a delay of 24 hours and was accompanied by an increase in IGFBP-2 from about 11 to 16 nmol/l i.e. about one fifth of the molar loss of the two ligands. On the other hand, Oldham and coworkers (1999) observed a decrease in plasma IGF-II in response to fasting only in neonatal sheep but increased concentrations in fasted mature animals accompanied by increases in IGFBP-2. Circulating IGFBP-2 was positively related to changes in IGF-II and negatively to IGF-I in sheep (Gallaher *et al.*, 1995).

While systemic IGF-II does not appear to have an important role in adult rodents (Holly, 1998), IGF-II is required for the regulation of glycogen metabolism during the perinatal period in mice, possibly via stimulation of glycogen synthase activity (Lopez *et al.*, 1999). Moreover, glucose, in the form of glucose-6-phosphate, stimulated synthesis and secretion of both IGF-I and IGF-II

by fetal rat hepatocytes in vitro (Goya *et al.*, 1999), suggesting the existence of feed-back mechanisms. Infusion of IGF-I into poorly fed beef heifers to preserve body protein reserves, besides reducing plasma amino acid and glucose (from about 3.5 to 1.3 mmol/l) levels, decreased plasma IGF-II from about 39 nmol/l to about 19 nmol/l within 8 hours accompanied by induction of putative IGFBP-1 (31-32 kDa). Thus, IGF-II appears to be subject to metabolic control in adult cattle. In this investigation marked differences in serum IGF-II level were found postpartum between groups of healthy cows given fresh green lucerne and those fed on ensiled lucerne hay (Table 3) accompanied by similar differences in postprandial insulin and NEFA concentrations. It may be supposed that the cows in Group H were able to derive a greater proportion of their nutrient requirements from the diet than those in Group P. This would be favoured by the prevailing environmental temperature and ration composition. Initial T3 and IGF-I concentrations were similar in both groups but showed opposite time-related trends. Namely, although the cows in Group P appeared to be successfully metabolising NEFA and maintaining blood glucose concentrations above 2 mmol/l, by day 4 postpartum both T3 and T4 were approaching the levels found in the group expressing ketonuria (Table 3).

Further investigations are needed to elucidate the role of the thyroid hormone axis in the appearance of spontaneous ketosis postpartum, since a negative correlation was found between serum T3 and the extent of fat accumulation in liver cells of ketotic cows (Samanc *et al.*, 2000). Our finding that serum T3 concentrations become independently related to IGF-I concentrations postpartum, in contrast to the equivalent independent relationship between T4 and IGF-I prepartum (Table 5), indicates an alteration of prevailing mechanisms controlling entrance and exit of these hormones from the serum pool. It may be speculated that, while the immediate thyroid hormone reserve (T4) is more important prepartum when nutrient supply may be assumed to be adequate or even in excess of requirements, deiodination mechanisms, particularly in the liver, assume the most important role postpartum. Namely, factors such as an adequate nutrient supply probably have similar effects on hepatic IGF-I synthesis and T4 deiodination.

Overall, this investigation documents for the first time the physiological decrease in circulating IGF-II associated with parturition, uncovers differences in IGF-II concentrations probably associated with nutritional factors and demonstrates the tendency towards constant total IGF concentrations in periparturient cows. The characteristic close association between thyroid hormone and IGF-I concentrations was preserved during nutrient excess prepartum and nutrient deficiency leading to ketosis postpartum.

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KONCENTRACIJE INSULINU-SLIČNIH FAKTORA RASTA I TIREOIDNIH HORMONA U SERUMU ZDRAVIH I KETOZNIH KRAVA U TOKU PUERPERIJUMA

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SADRŽAJ

Kod visokomlečnih krava, faktori rasta slični insulinu (IGF) i tireoidni hormoni (T3 i T4) igraju važnu ulogu u endokrinoj kontroli metabolizma u periodu pre i posle telenja. U prvom ogledu je nađeno da su se smanjile koncentracije T3, T4 i IGF-I u krvnom serumu u toku perioda od 7 dana pre telenja do 7 dana posle telenja. Stepen smanjenja je bio izraziiji u grupi krava (n = 24) sa prosečnom vrednošću telesnog kondicionog zbira (BCS) od 4.2 nego u grupi krava (n = 24) sa BCS vrednošću od 3.2. Serumske koncentracije IGF-II su se povećavale u ispitivanom periodu na način koji je održavao molarni zbir IGF-ova na relativno stabilnom nivou. U drugom ogledu, u rano proleće, uzimani su uzorci krvi od 12 zdravih krava svakog dana u intervalu od 4 dana pre telenja do 4 dana posle telenja i dokazane su slične promene sa statistički značajnim minimumom u koncentracijama IGF-II na dan telenja. U drugoj grupi zdravih krava (n = 12) koje su u maju kao deo obroka dobijale svežu zelenu lucerku, umesto siliranog

lucerkinog sena, koncentracije tireoidnih hormona i IGF-I su se povećavale u toku prva 4 dana posle telenja. Kod treće grupe krava (n = 12) koje su imale ketonska tela u mokraći nađene su uniformno niske koncentracije ovih hormona u istom intervalu. Najtesnija korelacija je konstatovane između koncentracija T3 i T4, a utvrdjena je i značajno nezavisna asocijacija izmedju nivoa T3 (odnosno T4) i koncentracije IGF-I u serumu ispitivanih krava. Karakteristični međusobni odnosi izmedju sva četiri hormona bili su očuvani u oba ogleda, što ukazuje da su veze između mehanizama koji kontrolišu njihove sinteze i degradacije sačuvane u nekim uslovima manjka ili viška hranljivih materija.