

LOW PERIPHERAL SERUM THYROID HORMONE STATUS INDEPENDENTLY AFFECTS THE HORMONE PROFILE OF HEALTHY AND KETOTIC COWS DURING THE FIRST WEEK POST PARTUM

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(Received, 19 December 1996)

Serum hormone concentrations were determined in twelve apparently healthy Holstein-Friesian cows and fourteen cows exhibiting the first signs of spontaneous ketosis a few days after parturition. Since about one third of the animals in each group were found to have low serum levels of thyroid hormones ($T_4 < 30 \text{ nmol/l}$ and/or $T_3 < 1.0 \text{ nmol/l}$), the results were analysed for four subgroups.

No differences between the main groups or subgroups were found concerning day of blood sampling, previous milk yield ($n = 22$), calf birth weight or serum insulin concentration. Blood glucose and serum progesterone concentrations were lower in the group of cows showing signs of ketosis than in the apparently healthy cows ($P < 0.05$) with no significant influence of thyroid hormone status. On the other hand, serum cortisol levels of thyroid hormones regardless of their energy status ($P = 0.07$). Total serum IGF-I concentrations were also similar in these subgroups of animals and lower than those in the healthy cows ($n = 8$). In the remaining ketotic cows ($n = 8$) serum thyroid hormone concentrations were within normal physiological limits but were significantly lower than in the subgroup of healthy cows ($P < 0.05$). Mean serum insulin and cortisol concentrations tended to be slightly lower, while mean IGF-I level showed a highly significant more than four-fold decrease in comparison with the control group.

It may be concluded that the appearance of ketosis and/or inadequate thyroid hormone status independently affect the serum hormone profile in puerperal cows. Namely, while energy deficiency alone or combined with low thyroid hormone levels is accompanied by lower blood glucose and progesterone levels, a low thyroid hormone status nullifies the effect of energy deficiency on total IGF-I concentrations and is accompanied by lower serum cortisol concentrations regardless of the energy status of the cows. Therefore, in this very sensitive postpartal period investigations on metabolic control in cows should include a wide selection of parameters for valid inferences to be made.

Key words: cows, ketosis, thyroid hormones, steroids, IGF-I

INTRODUCTION

Ketosis, characterized by high blood ketone levels and low blood glucose, is still a very real problem in early lactation in high yielding cows, because mechanisms which combat any lack of glucogenic energy in later stages of lactation by reducing milk output, fail to operate in the initial stages leading to a glucogenic energy crisis (Orskov, 1980; Nielsen and Riis, 1993). Of necessity, feed consumption by cows is low during parturition to be followed by a rapid and overriding requirement for glucose and aminoacids in the udder as colostrum and milk secretion starts. This is accompanied by massive mobilization of triacylglycerols and protein from body tissue and intensive gluconeogenesis in the liver, which are all processes under a complex system of hormonal control.

The partitioning of dietary and endogenous nutrients during lactation is not well understood even in man. Recently, it has been proposed that insulin and cortisol modulate the channelling of nutrients between anabolic and anti-anabolic aspects of maternal body protein metabolism, whereas thyroid hormones and cortisol modulate nutrient partitioning towards milk production and visceral protein synthesis (Motil et al., 1994). However, there are marked differences between monogastric animals, in which the supply of glucogenic energy is not compromised by microbial activity, and ruminants, as exemplified by postparturient insulin-like growth factor-I (IGF-I) levels (Schams et al., 1994). Namely, the clear increase at parturition and during lactation in cows was contrary to the secretion pattern found in cows where the lipolytic action of growth hormone (GH) was supported by a steep decrease of insulin and the uncoupling of IGF-I secretion from GH during the first weeks post partum (Schams et al., 1991; Hadsell et al., 1993; Hoshino et al., 1991). A moderate response to exogenous GH had returned at 3 weeks after calving (Vicini et al., 1991), although the uncoupling could be mimicked by feed deprivation in mid-lactation (McGuire et al., 1995a).

Kunz and Blum (1985) established that peripheral plasma concentrations of insulin, thyroxine (T₄) and triiodothyronine (T₃) were positively correlated with energy balance in dairy cows in the period from 5 to 70 days after parturition. Moreover, Shaw and coworkers (1975) increased milk production by feeding an iodinated casein containing 1% thyroxine to lactating cows. However, other authors found either no association between milk yield and thyroid hormone concentrations (Hoshino et al., 1991) or negative correlations at least for T₄ (Blum et al., 1983; Oldenbroek et al., 1989). Thyroxine is not appreciably excreted in the milk (about 1% of the total plasma content), whereas daily milk T₃ excretion may be considerable, amounting to about one third of the plasma content and highly correlated to plasma T₃ concentrations (Magdub et al., 1982). Triiodothyronine is the metabolically active thyroid hormone which influences organ and cellular metabolism, although its action may be modified by other hormones (e. g. Pellizas et al., 1966). The interactions between the different hypophyseal/peripheral endocrine organ axes are only slowly being elucidated, even though it was clear long ago, for example, that exogenous GH and adrenocorticotropin (ACTH) increased blood sugar in lactating cattle by different additive mechanisms.

Recently, attention has been paid to interactions between the GH/IGF-I axis and thyroid hormone action (Näntö-Salonen et al., 1993), insulin action (McGuire et al., 1995b) and adrenergic challenge (Houseknecht et al., 1995).

As a contribution to these studies on hormonal interactions in the regulation of metabolism in lactating cows, this paper discusses serum hormone concentrations found in the first week after calving in relation to the appearance of spontaneous ketosis.

MATERIALS AND METHODS

Animals and procedure. A total of 26 Holstein Friesian cows kept tethered in the maternity parlour on a large dairy farm were included in the investigation. Twelve of the cows were clinically healthy while fourteen had exhibited the first clinical signs of ketosis (inappetance, ruminal atony etc.).

Blood samples were obtained by jugular puncture at about 10⁰⁰ am approximately 3 hours after offering the morning portion of concentrate on average at 3 - 4 days after calving. All calves, of mean birth weight (SD) 38.8 (4.9) kg, had been born healthy and the mean yield of the 22 pluriparous cows in their first lactation was 6400 (876) litres milk (calculated for 305 days).

Analyses. After immediate direct determination of blood glucose concentrations using Dextrostix tracks, the blood samples were allowed to clot spontaneously at room temperature. The serum was then decanted, centrifuged at 300 rpm and preserved at - 18°C until analysed.

The hormones T3, T4, progesterone and cortisol were determined by liquid phase radioimmunoassay (RIA) using commercial kits in accordance with the instructions (INEP, Diagnostics, Zemun). Insulin was measured using a heterologous RIA system which included 125 I-porcine insulin, rabbit antibodies to porcine insulin and standard solutions of bovine insulin (Nikolić et al., 1989). Insulin-like growth factor-I was determined after separation of binding proteins using acid-ethanol and cryoprecipitation (Breier et al., 1991) as described and validated recently for our conditions (Nikolić et al., 1996). The mean intra-assay coefficients of variation of duplicate samples were as follows: T3 - 3.5%; T4 - 4.1%; progesterone - 5.0%; cortisol - 6.5% insulin - 6.6%; IGF-I - 8.0%.

Statistical analysis. Associations between the different parameters were sought by correlation analysis. The results were also subjected to analysis of variance after subgrouping each group of cows according to their thyroid status. Since Bartlett's test indicated heterogeneity of variance among the subgroups for all biochemical parameters except for T4 concentrations, logarithmic transformation of the data was carried out. Variance was then homogenous for each parameter except for glucose concentrations where the scatter was much greater in the ketotic subgroups than in the apparently healthy cows, even after logarithmic transformation. The statistical significance of differences between the means for the four subgroups was estimated by the least significant difference (LSD) test.

RESULTS

The results obtained are summarized in Table 1, where it can be seen that there were no significant differences between the main groups or subgroups of cows concerning day of blood sampling, previous milk yield and calf birth weight.

Table 1. Mean values for the variables determined in Holstein-Friesian cows (n = 26) separated into groups according to their energy and thyroid hormone status.

Variable	Group mean values					SE	F	P
	1	2	3	4				
	1 + 2		2 + 3		3 + 4			
Time after calving (days)	3.9	4.5	3.7	3.3	0.3	0.73	NS	
	4.1		3.4			1.44	NS	
Calf weight (kg)	36.1	41.5	38.0	40.9	1.0	1.89	NS	
	37.9		39.6			0.79	NS	
Milk yield in 1st lactation (l)*	6210	7115	6391	6165	187	1.19	NS	
	6612		6252			0.89	NS	
Glucose (mmol/l)	2.54 ^{ab}	2.83 ^a	2.09 ^{bc}	1.91 ^c	0.10	6.83	0.002	
	2.64 ^a		1.99 ^b			18.04	0.0003	
T4 (nmol/l)	50.8 ^a	21.7 ^c	24.5 ^c	41.0 ^b	2.8	18.82	< 0.0001	
T3 (nmol/l)	2.73 ^a	1.08 ^c	0.93 ^c	1.83 ^b	0.17	15.97	< 0.0001	
Log transformation	a	c	c	b		18.87	< 0.0001	
Progest (nmol/l)	1.15 ^{ab}	1.97 ^a	0.88 ^b	0.82 ^b	0.14	3.58	0.030	
	1.42 ^a		0.85 ^b			5.28	0.031	
Log transformation	a		b			6.16	0.020	
Cortisol (nmol/l)	46.5	19.6	15.9	34.3	5.4	1.92	NS	
	46.5 ^a	17.4 ^b		34.3 ^{ab}		2.99	0.070	
Log transformation	a	b		ab		2.55	0.100	
Insulin (mIU/l)	38.4	48.5	34.4	20.4	5.7	0.99	NS	
IGF-I (μg/l)	77.0 ^a	50.4 ^{ab}	42.9 ^{bc}	17.8 ^c	6.3	8.79	0.0005	
	77.0 ^a	45.9 ^b		17.8 ^c		13.51	0.0001	
Log transformation	a	a		b		20.96	< 0.0001	

Subgroup 1 - Healthy cows with normal thyroid status (n = 8)

Subgroup 2 - Cows with low T3 and T4 levels (n = 4)

Subgroup 3 Ketotic cows with low T3 and T4 levels (n = 6)

Subgroup 4 Ketotic cows with normal thyroid status (n = 8)

a, b, c, Groups described by the same letter do not differ significantly (LSD test at P < 0.05 and n = 6 for four groups or n = 8 or 9 for three groups)

* n = 22

Since four cows among the apparently healthy animals and six of those showing the first signs of spontaneous ketosis were found to have low serum levels of thyroid hormones (T4 < 30 nmol/l and/or T3 < 1.0 nmol/l), each main group of cows was subdivided into groups with normal or low thyroid hormone status, because the concentrations of certain hormones appeared to be associated with

thyroid status as well as or rather than with ketotic or normal glycemic state. Namely, despite the large variance in both subgroups of ketotic cows, mean blood glucose concentrations were lower in each subgroup of ketotic cows than in the corresponding subgroup of healthy cows (Table 1; subgroup 1 > 4 and 2 > 3). In each case mean blood glucose levels of the low thyroid hormone subgroup were marginally greater than in the subgroups with normal thyroid hormone status.

The thyroid hormones themselves exhibited marked differences in serum concentration between the subgroups. Both T3 and T4 concentrations were the highest in the subgroup of eight healthy cows (subgroup 1), followed by significantly lower but still adequate concentrations in eight cows exhibiting ketosis (subgroup 4). The two subgroups with low thyroid hormone status did not differ from each other but serum T3 and T4 concentration were significantly lower in each subgroup than in each of the other groups (Table 1).

Serum cortisol concentrations differed more widely between animals but,

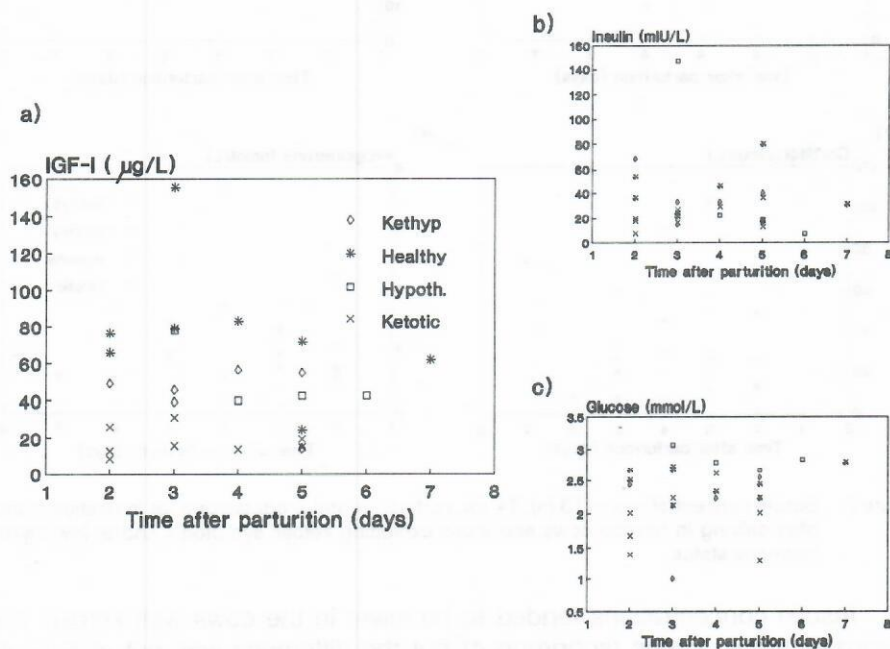


Figure 1. Peripheral concentrations of IGF-I (a), insulin (b), and glucose (c) in relation to time after calving in healthy cows and those exhibiting ketotic symptoms and/or low thyroid hormone status.

groupwise, appeared to be more associated with thyroid hormone than with glycemic status. Thus, there was a tendency for cortisol concentrations to be highest in the healthy cows with normal thyroid hormone levels and lowest in the cows with low levels of serum T3 and T4 (Table 1). When these cows were placed

in a single group ($n = 10$) and analysis of variance carried out for three groups, the difference between the mean value for healthy cows and that for possibly hypothyroid cows approached statistical significance ($P = 0.07$). These apparent differences need to be confirmed with a larger number of animals.

On the other hand progesterone concentrations showed statistically significant differences between healthy and ketotic groups of cows and no apparent association with thyroid hormone status.

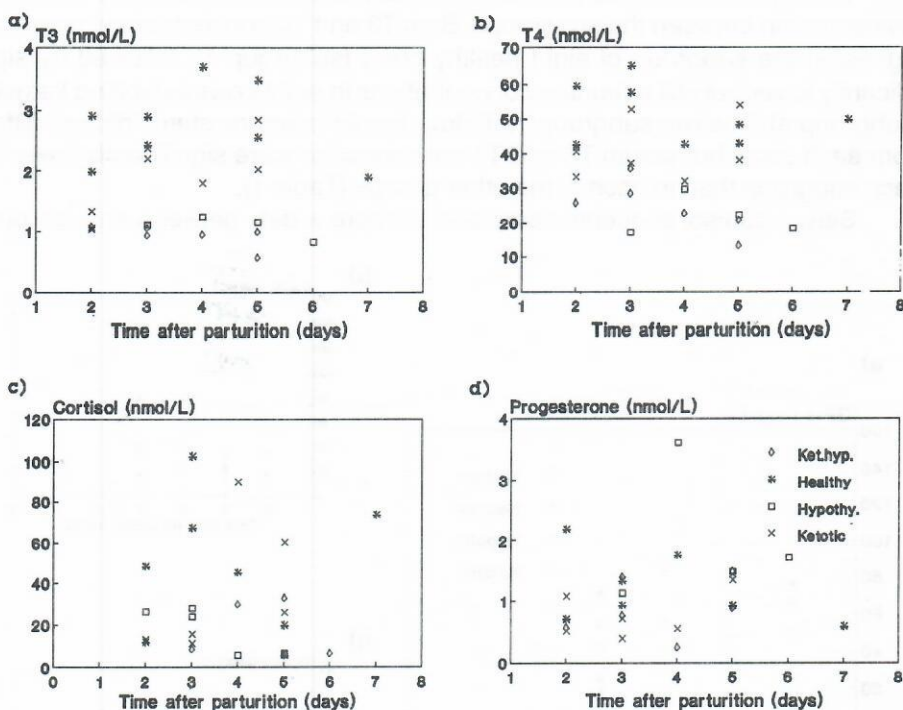


Figure 2. Serum concentrations of T3 (a), T4 (b), cortisol (c) and progesterone (d) in relation to time after calving in healthy cows and those exhibiting ketotic symptoms and/or low thyroid hormone status.

Insulin concentrations tended to be lower in the cows with ketosis and adequate thyroid status (subgroup 4) but the difference was not statistically significant (Table 1).

However, serum total IGF-I concentrations were markedly different in the different subgroups of cows. The highest mean value was observed for healthy cows with normal thyroid hormone levels (subgroup 1). The concentrations were somewhat lower in the subgroups of cows with low serum T3 and T4, the difference being significantly different for plain but not for logarithm transformed data (Table 1). Mean IGF-I levels were similar in both subgroups with low thyroid

hormone levels. The mean serum concentration of IGF-I was significantly lower in the subgroup of ketotic cows with normal thyroid hormone level than in each of the other subgroups.

Although none of the seven biochemical parameters appeared to change with time after parturition, individual results for the 26 cows are shown in Figures 1 and 2, where the differences summarized in Table 1 become evident. Only three statistically significant correlations between the parameters were observed, the closest being between T3 and T4 concentrations ($r = 0.703$; $P < 0.0001$). Thyroid hormones also tended to be positively correlated with cortisol concentrations ($r = 0.391$; $P = 0.048$ and $r = 0.345$; $P = 0.084$ for T4 and T3 respectively). The third statistically significant correlation was between blood glucose and serum IGF-I concentrations ($r = 0.421$; $P = 0.032$) which probably reflects the important role of GH in the immediate postpartal period.

DISCUSSION

The finding of a statistically significant correlation between blood glucose and serum IGF-I concentrations rather than serum insulin concentrations in the present investigation may relate to the fact that serum total IGF-I represents primarily the circulating store of IGF-I bound to several specific proteins and comparable in function to the granules containing insulin stored in pancreatic beta cells. Circulating insulin is metabolically active and present in molar concentrations similar to the free fraction of IGF-I. Using propionate loading or glucose tolerance tests it can be shown that the insulin secretion response is lower in spontaneously ketotic cows than in healthy cows (Šamanc et al., 1996). Namely, it appears that two branches of the reserve anabolic signal are diminished in the ketotic state.

The metabolic energy deficit was also reflected in lower thyroid hormone levels in the ketotic cows but the difference was much less pronounced than the difference in IGF-I levels. Besides being decreased in energy deficient states (Blum and Kunz, 1981), thyroid hormone concentrations are also dependent on the supply of inorganic iodine (Aumont et al., 1989; Symonds, 1995). Iodide is also excreted in the milk representing a sudden new drain for the postparturient cow. It seems that about one third of the cows in this investigation may have been suffering from a functional iodine deficiency which was separate from their tendency to become ketotic or not. The very low thyroid hormone status was accompanied by lower but similar serum cortisol concentrations regardless of the glycemic status, which confirms the conclusion of Ely and Baldwin (1976) that cortisol is not one of the major hormones affecting gluconeogenesis in ruminant liver. Hepatic metabolism of propionate and glucogenic amino acids into glucose is probably regulated primarily by the availability of the substrates and glucagon (Danfaer et al., 1995) which are both compromised in Ketotic cows (Bruss et al., 1986).

Glucocorticoids, catecholamines and thyroid hormones promote protein degradation by different mechanisms (Grizard et al., 1995) whereas insulin, IGF-I and thyroid hormones have anabolic actions. In this investigation it seems that the association between thyroid hormone status and IGF-I levels is independent

of the effect of ketosis, because IGF-I levels in both subgroups of cows with low thyroid hormone status were intermediate between the healthy and the ketotic groups with normal thyroid status. These differences are logical if one accepts that overall metabolic turnover rate with its associated energy losses is dependent on thyroid hormone levels. When these are low the balance of mainly catabolic and mainly anabolic hormones is set at a lower point in the healthy animals (i. e. lower IGF-I and cortisol levels), whereas in the energy deficient state the accompanying low thyroid hormone levels provide some kind of protection from energy loss, permitting relatively high IGF-I and relatively low cortisol levels in comparison with the subgroup in which thyroid hormone concentrations were in the normal range and in which net energy expenditure and therefore tissue mobilisation would be expected to be greater.

The serum concentrations of the hormones determined here confirm the values detected in healthy lactating cows by other authors (Bitman et al., 1994; Wagner and Oxenreider, 1972; Convey, 1974). No differences were found between IGF-I levels in heifers and cows which contrasts with the results of Kerr and coworkers (1991). At any one time serum hormone concentrations represent the balance between synthesis and utilisation but give no measure of the rate at which these processes occur. Moreover, among the hormones determined here only insulin circulates in a predominantly active form, the action of the others being modified by specific and non-specific binding proteins which may be present in different relative amounts at a given concentration and may have a variety of influences on hormone availability. Nevertheless, these preliminary results concerning a small number of animals illustrate some associations and interrelationships between prevailing hormone levels and energy deficit as manifested by the ketotic state accompanied or not by low thyroid hormone status. Further investigations will be aimed at relating these phenomena to nutrient intake.

This work was supported by the Ministry of Science and Technology of the Republic of Serbia.

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J. Anna Nikolić et al.: Low peripheral serum thyroid hormone status independently affects the hormone profile of healthy and ketotic cows during the first week post partum.

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ISPITIVANJE UTICAJA NISKIH KONCENTRACIJA TIREOIDNIH HORMONA U SERUMU NA HORMONSKI PROFIL ZDRAVIH ODNOSNO KETOZNIH KRAVA U TOKU PRVE NEDELJE POSLE TELENJA

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SADRŽAJ

Određene su koncentracije hormona u krvnom serumu dvanaest prividno zdravih krava holštajn-frizijske rase kao i kod četrnaest krava koje su ispoljavale prve simptome spontane ketoze nekoliko dana posle telenja. Pošto su niski nivoi tireoidnih hormona ($T_4 < 30 \text{ nmol/l}$ i/ili $T_3 < 1.0 \text{ nmol/l}$) nađeni u serumu oko trećine životinja u obe grupe, rezultati su podeljeni u četiri podgrupe za statističku analizu.

Nisu postojale razlike između glavnih grupa odnosno podgrupa krava u pogledu dana uzimanja uzoraka krvi, prinosa mleka u prvoj laktaciji ($n = 22$), telesne težine teleta pri rođenju i koncentracije insulina. Koncentracije glukoze u krvi i progesterona u serumu su bile niže u grupi ketoznih krava nego u grupi prividno zdravih krava ($P < 0.05$) bez uticaja od strane nivoa tireoidnih hormona. Nasuprot tome, nivoi kortizola su pokazali tendenciju da budu niži u podgrupama krava sa niskim nivoima tireoidnih hormona nezavisno od njihovog energetskeg stanja ($P = < 0.07$). Koncentracije ukupnog IGF-I su isto tako bile slične u tim podgrupama, a niže od onih kod zdravih krava ($n = 8$). Nađeno je da su koncentracije tireoidnih hormona kod ostalih ketoznih krava ($n = 8$) bile unutar normalnog fiziološkog opsega, mada bile su značajno niže od onih u podgrupi zdravih krava ($P < 0.05$). Pored toga, prosečni nivoi insulina i kortizola su pokazali tendenciju da budu nešto niži, dok je prosečni nivo IGF-I u serumu ispoljavao vrlo

značajno (više od četiri puta) smanjenje u odnosu na vrednost za kontrolnu grupu.

Možemo da zaključimo da pojava ketoze i/ili neadekvatni status tireoidnih hormona utiču nezavisno na profil hormona u serumu peripartalnih krava. Naime, dok je deficit energije sam ili zajedno sa niskim nivoima tireoidnih hormona praćen niskim nivoima glukoze i progesterona, nizak nivo tireoidnih hormona poništava uticaj deficita energije na nivoima IGF-I. Pri tome, ovo stanje je praćeno niskim nivoima kortizola bez obzira na stanje energetskeg statusa krava. Ovo znači da u ovom vrlo osetljivom intervalu posle telenja, treba uključiti što veći broj parametara u istraživanja posvećena kontroli metabolizma krava da bi se izveli pravi zaključci.

