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Thyroxine in Blood Plasma Related to Plasma Levels of Acetoacetate and Glucose in Ketotic and Healthy Cows

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Ropstad E., K. Halse and A. O. Refsdal: Thyroxine in blood plasma related to plasma levels of acetoacetate and glucose in ketotic and healthy cows. Acta vet. scand. 1989, 30, 175–183. – Plasma samples were taken before morning feeding twice weekly for the first 3 months post partum from 28 individually fed cows, of which 8 developed clinical ketosis. Feed rations consisted of grass silage ad libitum and concentrates. The high incidence of ketosis may be accounted for mainly by substandard amounts of feed energy, and by variations in the quality of the grass silage.

The following sources of post-partum thyroxine variation were found to be statistically significant ($p < 0.001$): The individual cow, the individual pre-calving thyroxine level, metabolic status estimated by plasma levels of acetoacetate or glucose, and elapsed time from partus. The post-partum decrease in thyroxine was greater in cows which developed ketosis (55 %) than at the corresponding stage of lactation in those which remained moderately ketonaemic (33 %). Extensive overlapping of ranges nevertheless indicates that the apparent hypothyroidism of ketosis is of limited importance for the etiology of the disease. Susceptibility to ketosis was not related to the thyroxine levels observed before partus or after recovery from the disease, in the third month of lactation. Evidence was obtained of a common glucose threshold of about 3.6 mmol/l, below which decreasing sugar levels were accompanied by progressive increments in acetoacetate and decreases in thyroxine. Above the threshold level, the average level of the hormone increased from the first to the third month of lactation in spite of a poor correlation to glucose.

early lactation; pre-feeding levels; ketosis.

Introduction

Several studies have shown that the plasma concentrations of thyroxine are depressed in early lactation in dairy cows (*Mixner et al.* 1962, *Heitzman & Mallinson* 1972, *Hart et al.* 1978, *Thilsted* 1985). Levels of thyroid hormones correlate positively with energy intake (*Davidson & Chopra* 1979, *Blum et al.* 1980, *Riis & Madsen* 1985), and cows suffering from clinical ketosis have very low thyroxine levels (*Heizman & Mallinson* 1972, *Tveit et al.* 1980).

When sensitive methods of detection are employed and sampling takes place before morning feeding, moderate ketonaemia accompanied by decreases in blood sugar is observed in early lactation in many cows which remain free of ketosis symptoms (*Halse & Mogstad* 1975). In such animals, gradual stabilization of levels within narrow ranges of plasma ketones (acetoacetate) and glucose usually takes place during the second to third month of lactation. Evidently, the energy metabolites show variations

which coincide with lactation-induced variations in thyroxine. In the present study the strength of the correlation between thyroxine and the mentioned indicators of carbohydrate and fat metabolism has been tested, and compared to effects from other potential sources of hormone variation.

Materials and methods

Animals and feeding

Twenty-eight Norwegian Red Cattle dairy cows were randomly assigned to a 2×2 factorial feeding experiment. The cows were fed grass silage ad libitum and concentrates according to yield and experimental group. The groups differed in protein and energy levels. Two batches of concentrates were made, one with high (Hp) and one with low (Lp) protein content (17.5 % digestible crude protein (DCP) and 12.5 % DCP, respectively). Within each protein content 2 levels of energy supply were used (He and Le).

Seven of the 21 multiparous cows had to be treated for ketosis (4 in the Le groups and 3 in the He groups). In addition, one heifer developed ketosis 66 days post partum (Le group). Cows which developed ketosis were treated with 150 mg prednisolone acetate, given intramuscularly. In addition, oral supplements of sodium propionate were given for 5 days after treatment. Further information about animals and feeding is given by *Ropstad et al.* (1989).

Blood samples

Blood samples were collected twice weekly from the jugular vein before morning feeding at 6 a. m., starting at about 2 weeks before, and continuing until 12 weeks after calving. Heparinized vacutainers were used, and the plasma produced by centrifugation immediately after sampling was stored either in liquid nitrogen (–196°C) for the determination of acetoacetate and glucose, or at –20°C for the thyroxine assay.

Plasma acetoacetate (ACAC) and glucose levels were determined by the methods described by *Blom & Halse* (1975), while the plasma concentration of total thyroxine was determined by radioimmunoassay as described by *Larsen et al.* (1973), with the modification introduced by *Andresen et al.* (1980). The properties of the antiserum used have previously been described by *Kruse* (1976).

Statistical analysis

Statistical analyses were performed using conventional t-tests, correlation tests and variance analyses i.e. the GLM procedure from Statistical Analysis System (*SAS* 1982). The levels of plasma thyroxine were evaluated by linear models which accounted for the effects of the animal factor (COW), the week of lactation (1, 2 . . . 12) and metabolic condition indicated either by plasma ACAC levels (Model A) or plasma glucose levels (Model B). Alternatively the animal factor was replaced by the individual pre-calving thyroxine level and lactation number (1 or > 1) (Models C and D).

Results

Ketonaemia and ketosis

One cow treated for ketosis 34 days after calving was analytically a borderline case with plasma ACAC as low as 0.58 mmol/l. The remaining 7 ketotic animals had ACAC values at treatment in the range 1.8–3.23 mmol/l (corresponding glucose mean ± SD: 2.48 ± 0.27 mmol/l).

The pre-parturient levels of ACAC, glucose and thyroxine in cows which developed ketosis were not significantly different from those of non-ketotic cows (Table 1). However, after 7–10 days of lactation, the 6 cows

Table 1. Comparison of plasma levels of acetoacetate, glucose and thyroxine at three stages of the lactation cycle in cows which did (+) and did not (–) develop clinical ketosis. a: first treatments and relapsed cases, b: observations representing the same days post partum as in material a.

	Time	Ketosis	Number of cows	Number of samples	Mean ± SD Acetoacetate mmol/l	Mean ± SD Glucose mmol/l	Mean ± SD Thyroxine nmol/l
I	3–12 days before partus	+	8	24	0.024 ± 0.012	4.12 ± 0.16	67.6 ± 14.3
		–	19	47	0.023 ± 0.009	4.18 ± 0.22	71.3 ± 20.5
II	11–66 days after partus	+ a	8	14	1.69 ± 0.97	2.68 ± 0.31	30.5 ± 12.9
		– b	20	140	0.11 ± 0.12	3.97 ± 0.36	47.7 ± 12.8
III	70–92 days after partus	+	8	48	0.053 ± 0.05	4.14 ± 0.24	58.5 ± 16.4
		–	20	144	0.035 ± 0.016	4.14 ± 0.28	59.55 ± 17.2

Thyroxine, t-tests: I–IIa. $p < 0.001$, I–IIb $p < 0.01$, II b–a $p < 0.01$, III–II a and b $p < 0.01$.

which developed the disease within the first 3 weeks post partum showed ACAC and glucose averages ± SD of 0.33 ± 0.19 and 3.3 ± 0.25, respectively, as opposed to 0.09 ± 0.11 and 3.9 ± 0.4 (mmol/l) measured at the same stage in 8 multiparous cows which remained healthy in the same feeding groups.

Sources of thyroxine variation

Significant effects of the metabolic condition of the animals on the hormone level during the first 3 months post partum were obtained both with ACAC and glucose as indicators (Table 2, $p < 0.001$). Additionally, significant variance contributions were obtained from week of lactation as an independent variable. The largest source of variation was, however, the individual cow, giving rise to about 45 % of the total variance. More or less permanent differences between individuals were indicated by the fact that more than 12 % of the variance observed post partum could be explained by individual variations before partus (Table 2). The persistence of such differences was further evidenced by the finding of a significant correlation between individual thyroxine averages obtained before partus and after 70–90 days of lactation ($r = 0.52$, $n = 27$, $p < 0.05$). The constancy of ACAC and glucose between the 2 time intervals compared is indicated in Table 1.

Thyroxine during ketosis

The hormone average was reduced to 45 % of the pre-parturient level at the time of ketosis treatment, and was significantly lower than in healthy controls sampled at the same stage of lactation (Table 1, observations from healthy controls were weighted according to the post partum timing of the ketosis cases). The previously mentioned ketone maxima shown by the 7 severely ketonaemic patients (excluding the borderline case) was associated with a plasma thyroxine

Table 2. Analysis of variance. effects of week of lactation, cow, individual precalving thyroxine level, number of lactation and energy metabolite levels on the variation of thyroxine. Twenty eight cows were sampled twice weekly during the first 3 months of lactation.

	Per cent of total sum of squares and level of significance*			
Independent variables	Model A	Model B	Model C	Model D
Week of lactation	7.3^{c}	6.6^{c}	6.4^{c}	5.4^{c}
Cow	45.9^{c}	45.3^{c}	–	–
Pre-calving thyroxine level	–	–	13.6^{c}	12.8^{c}
Plasma level of ACAC**	4.0^{c}	–	9.1^{c}	–
Plasma level of glucose	–	4.1^{c}	–	10.0^{c}
Number of lactation	–	–	1.0$^{n\,s}$	0.4$^{n\,s}$
Model	65.0^{c}	65.0^{c}	41.7^{c}	42.8^{c}

* (Type III sum of squares/total sum of squares) × 100; by SAS (1982); PROC GLM
Level of significance. c: $p < 0.001$ n.s.: not significant
** ACAC = acetoacetate
Number of lactation = 1 or > 1

average as low as 23.6 ± 9.15 nmol/l (± SD).

Relationship between acetoacetate and thyroxine

Low thyroxine averages coincided with ACAC maxima before day 40 after calving (Table 3). Increments in thyroxine were associated with the subsequent stabilization of ACAC at low levels. In addition, the two parameters were found to be significantly negatively correlated within 10-day intervals until day 60 post partum.

Thyroxine ranges

Evidently the narrowing of the ketone range

Table 3. Plasma acetoacetate, thyroxine and coefficients of correlation between the two parameters related to stage post partum.

Days after calving	n	Mean ± sd				Range	
		Acetoacetate mmol/l	Thyroxine nmol/l	r_s	Sign level p	Acetoacetate mmol/l	Thyroxine nmol/l
0–10	77	0.100 ± 0.140	51.8 ± 19.6	–0.23	<0.05	0.020–0.430	20–117
11–20	79	0.310 ± 0.500	44.8 ± 14.9	–0.34	<0.01	0.016–2.750	16– 96
21–30	72	0.300 ± 0.470	44.9 ± 13.9	–0.33	<0.01	0.019–2.930	17– 74
31–40	71	0.350 ± 0.640	45.0 ± 13.1	–0.57	<0.001	0.018–3.230	15– 72
41–50	71	0.170 ± 0.310	53.4 ± 17.1	–0.23	<0.05	0.019–1.670	16– 90
51–60	74	0.170 ± 0.180	52.3 ± 12.6	–0.30	<0.01	0.016–0.860	25– 86
61–70	67	0.110 ± 0.290	53.8 ± 15.9	–0.16	n.s.	0.014–2.200	24– 84
71–80	76	0.04 ± 0.020	61.6 ± 17.0	0.11	n.s.	0.015–0.130	21–102
81–90	70	0.044 ± 0.040	58.8 ± 17.2	0.11	n.s.	0.011–0.240	24–130
>90	13	0.038 ± 0.015	60.2 ± 30.0	–0.10	n.s.	0.018–0.070	25–150

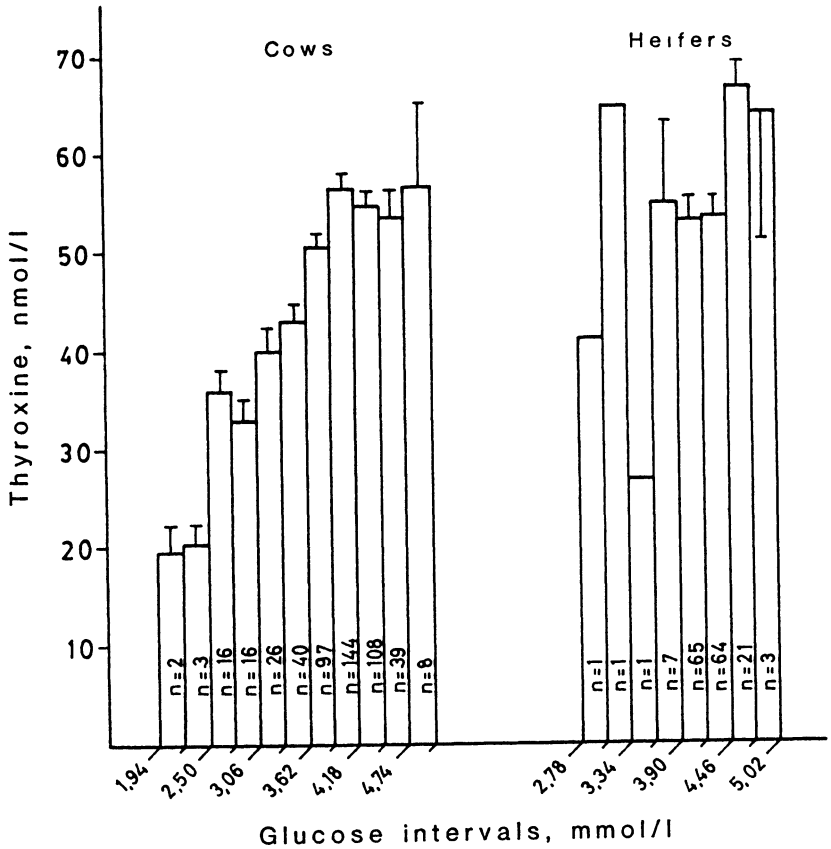

Figure 1. Plasma thyroxine (mmol/l) related to glucose levels in cows and heifers. The thyroxine values were averaged within glucose intervals of 0.28 mmol/l (n = number of observations within each interval). Standard errors are indicated.

in the third month of lactation was not followed by a comparable narrowing of the range of thyroxine (Table 3). Actually, hormone minima below the ketosis average in Table 1 were observed at this stage of lactation while all animals were low in ACAC. This finding should be considered in conjunction with the failure to detect differences in the variability of thyroxine before partus and in the third month of lactation between ketotic and non-ketotic cows (Table 1).

Simultaneous effect of glucose on thyroxine and acetoacetate

Fig. 1 and 2 show that decreases in plasma glucose below a level of about 3.6–3.9 mmol/l were accompanied by progressive decreases in the thyroxine mean and progressive increments in ACAC. Some threshold effect is indicated by the fact that thyroxine was correlated to glucose with $r = 0.47$, and to ACAC with $r = 0.48$ ($p < 0.001$) when glucose was below 3.9 mmol/l

Table 4. Plasma thyroxine concentrations at different periods after calving in cows with glucose levels below and above 3.6 mmol/l.

Days after calving	Thyroxine, nmol/l				Sign level p
	n	Glucose < 3,6 mmol/l	n	Glucose > 3 6 mmol/l	
0–10	13	43.1 ± 14.6	43	50.8 ± 19.9	n.s.
11–20	28	39.7 ± 13.7	31	45.8 ± 11.2	n.s.
21–30	19	34.8 ± 13.0	36	48.4 ± 11.2	< 0.001
31–40	16	32.4 ± 9.3	39	48.8 ± 11.0	< 0.001
41–50	12	37.8 ± 13.4	42	54.6 ± 13.4	< 0.001
51–60	6	38.3 ± 9.4	53	53.2 ± 11.1	–
61–70	3	33.3 ± 7.5	46	54.4 ± 14.7	–
71–80	3	62.6 ± 18.0	54	62.0 ± 15.3	–
81–90	3	49.0 ± 8.5	48	60.3 ± 18.1	–

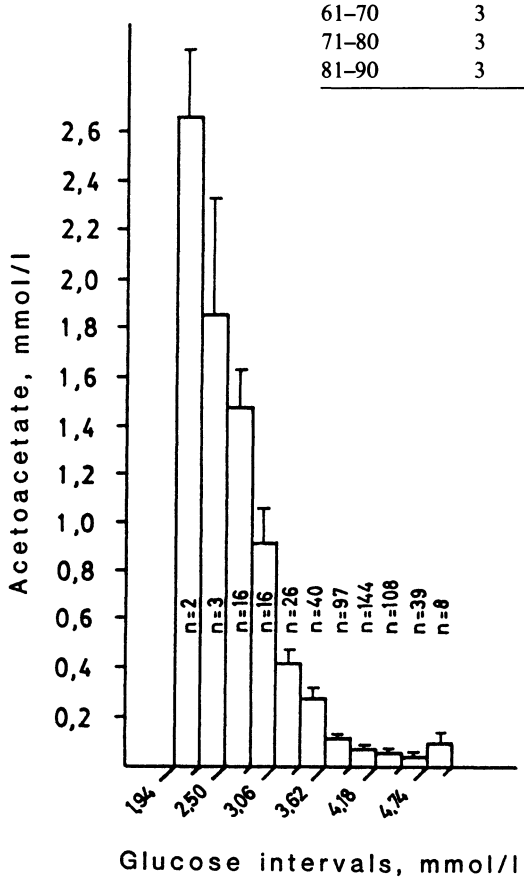

Figure 2. Mean levels of acetoacetate in cows related to glucose levels. Acetoacetate values were averaged within glucose intervals of 0.28 mmol/l (n = number of observations within each interval). Standard errors are indicated.

(n = 200). Much lower correlations to the 2 parameters (r = 0.04, n.s. and 0.14, p < 0.01, respectively) were obtained when glucose was above 3.9 mmol/l (n = 460).

Time effects unrelated to glucose

In accordance with the findings above, differences were obtained within 10-day intervals post partum between thyroxine averages representing glucose levels above and below 3.6 mmol/l (Table 4). The 25 % increase in thyroxine from the first to the end of the third month of lactation at glucose levels > 3.6 mmol/l was significant with p < 0.001 (Table 4). The same level of significance was obtained after further narrowing the glucose range to 3.9–4.4 mmol/l (increment 23 %).

Discussion

As reported by others (*Heitzman & Mallinson* 1972, *Tveit et al.* 1980). The ketotic thyroxine level was strongly decreased (Table 1). However, by following the animals with frequent observations for as long as 3 months, extensive overlapping was found between the hormone ranges during ketosis and after return to low ketone levels (days 70–90 in Table 3). This phenomenon, consistent with the large cow-effects in

Table 2, cannot be explained by persistence of the difference between ketotic and healthy cows (Table 1). The indication is that the hypothyroidism of ketosis is of limited importance in the etiology of the disease.

The persistence of individual differences in thyroxine detected before partus (Table 2) suggests the existence of sources of variation unrelated to the stresses of lactation. The phenomenon could not be explained by age effects (Table 2). Possibly differences exist between individuals in the plasma level at which the tissues become optimally supplied with active hormone metabolites.

Thyroxine variations unrelated to ACAC, glucose or the animal factor are indicated in Table 2 (week of lactation). An impression of the magnitude of the apparent metabolite-independent effect is provided by the average corresponding to glucose > 3.6 mmol/l in Table 4. Presumably, thyroxine will be influenced by lactation stresses which, according to circumstances and the condition of the animal, may or may not produce hypoglycaemia and ketonaemia. Thyroxine variations during lactation may otherwise be part of complex endocrine adaptations which secure the diversion of energy substrates to the synthesis of milk constituents. Attention is in this connection drawn to evidence from the literature of galactorrhea in women with primary hypothyroidism associated with increased prolactin levels (*How & Bewsher* 1979).

Fig. 1 provides evidence of effects on thyroxine which are related to variations in glucose < 3.6 – 3.9 mmol/l, i.e. above the range for clinical ketosis. This finding is consistent with the demonstration of decreases in thyroxine associated with increments in ACAC as moderate as from < 0.05 to 0.1–0.3 mmol/l by *Tveit et al.* (1980). Seemingly, by sampling in the morning, interrelations are detected which might be obscured by feeding-induced diurnal ketone and glucose variations at other hours of the day. The informative value of prefeeding sampling is illustrated by the accuracy with which prepartum glucose levels were reproduced 3 months later (Table 1). Attention is drawn to the correspondence with previous findings, using the same analytical methods, for cows with low ACAC levels (< 0.05 mmol/l): 4.1–4.2 (SD ± 0.3) mmol glucose before feeding (*Halse et al.* 1983).

For the interpretation of the findings in Figs. 1 and 2, it is of interest that analogous interrelationships between triiodothyronine and ketones and glucose have been observed in obese humans on carbohydrate-free, low-calory diets with r-values close to those observed in the present study (*Spaulding et al.* 1976). The correlation obtained for cows may reflect endocrine adaption to the lactation drain on carbohydrate reserves.

Thyroxine was reported to remain unaffected while triiodothyronine was low in the experiments reported by *Spaulding et al.* (1976). In contrast, the two hormones were found to be positively correlated in lactating cows (*Tveit et al.* 1980). Seemingly, thyroxine is a better indicator of thyroid hormone activity in bovines (*Blum et al.* 1979) than in man (*Palmblad et al.* 1977).

As may frequently be the case in the field, imbalance between milk yield and feed supply at the initiation of lactation seems to have been the main cause of ketosis in the present study. Systematic underfeeding after the second week of lactation may account for one late case of ketosis (day 66 post partum), for the high relapse rate and for the moderate, subclinical ketosis in cows in the Le-group. Otherwise, since the calvings were widely dispersed in time, the possibility can not be excluded of affects from variations in the quality of the grass silage on the susceptibility to ketonaemia and ketosis.

On the basis of the present results, and on evidence from the literature, it would seem appropriate to carry out further experiments to test the influence of antiketogenic supplements, such as proprionate, on the thyroxine level of cows on low-energy, ketonaemia-producing diets.

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Sammendrag

Tyroksin i plasma relatert til plasma nivåer av acetoacetat og glukose hos kyr med og uten ketose

Det ble tatt blodprøver før morgenfôring 2 ganger i uken de første 3 måneder etter kalving hos 28 individuelt fôrede kyr. Åtte kyr utviklet klinisk ketose. Kuene ble fôret med grassurfôr etter appetitt og kraftfôr. Den høye ketoseinsidensen var hovedsakelig forårsaket av underfôring med kraftfôr og varierende surfôrkvalitet.

Ved variansanalyse ga følgende faktorer et signifikant ($p < 0.001$) bidrag til variasjonen i tyroksin: Individ, det individuelle tyroksinnivå før kalving, metabolsk tilstand indikert ved plasma acetoacetat eller glukose og tidsavstand fra kalving.

Fallet i tyroksin etter kalving var større hos kyr som utviklet ketose (55 %) enn hos kyr som hadde moderat ketonemi i samme laktasjonsstadium (33 %), men den store variasjon i tyroksinnivåer indikerer at nedsatt tyroksinnivå ved ketose betyr lite for utvikling av sykdommen.

Det var ingen tendens til at kyr som hadde utviklet ketose hadde avvikende tyroksinnivåer før kalving eller etter helbredelse i 3. laktasjonsmåned. Det ble funnet holdepunkter for at blodsukkernivåer under en viss terskelverdi (ca. 3.6 mmol/l) var forbundet med progressiv økning av plasma ACAC og senkning av tyroksinnivåene. Over dette terskelnivået økte gjennomsnittlige tyroksinverdier fra første til tredje laktasjonsmåned til tross for en lav korrelasjon med glukose.

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