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Original article

Possible modulation of adipose tissue responsiveness to catecholamines by available dietary protein in dairy cows during early lactation

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Summary — Early lactation is characterised by a number of metabolic adaptations which divert nutrients from the peripheral tissues in favour of the mammary gland. One such adaptation is the increased responsiveness of adipose tissue to lipolytic stimuli. The effects of the condition score at calving and the level of undegradable protein in the diet on the responsiveness of adipose tissue to adrenergic stimulation were studied in dairy cows during early lactation. They were subjected to exogenous epinephrine challenges on weeks 3 and 8 of lactation. The responsiveness to epinephrine increased with high levels of rumen undegradable protein in the diet and a high condition score at calving, with no interaction between the 2 factors. The responsiveness to epinephrine was increased by the dietary treatment, especially on the 8th week of lactation, which suggests that the availability of protein might prolong the metabolic adaptations characteristic of early lactation.

lipolysis / early lactation / undegradable protein / condition score / epinephrine challenge

Résumé — Possible modulation de la réponse du tissu adipeux aux catécholamines par les protéines disponibles de la ration chez la vache laitière. *Le début de la lactation est caractérisé par de nombreuses adaptations métaboliques qui contribuent à détourner les nutriments des tissus périphériques vers la glande mammaire. L'une de ces adaptations correspond à la réponse croissante du tissu adipeux aux stimulus lipolytiques. Les effets de l'état d'engraissement au vêlage et du taux de protéines non dégradables dans la ration sur la réponse du tissu adipeux aux stimulations adrénergiques, ont été étudiés au début de la lactation chez des vaches laitières soumises à des infusions d'épinéphrine dans les semaines 3 et 8 de lactation. La réponse à l'épinéphrine a augmenté avec les taux élevés de protéines non dégradables dans le rumen et avec les valeurs élevées de l'état d'engraissement, sans interaction entre les 2 facteurs. La réponse à l'épinéphrine a augmenté avec le traitement alimentaire spécialement dans la semaine 8 de lactation. Cela suggère que la disponibilité de la protéine pourrait prolonger les adaptations métaboliques caractéristiques du début de lactation.*

lipolyse / début de lactation / protéine non dégradable / état d'engraissement / infusion d'épinéphrine

INTRODUCTION

Absorbable protein is probably the principal limiting nutrient in dairy cows during early lactation because the body protein reserves are relatively low and are more slowly mobilised than the fat reserves. It has been shown that supplying large proportions of absorbable protein significantly increases milk yield and accelerates body fat mobilisation (Ørskov *et al,* 1977; Rulquin, 1982). Calorimetric studies (Whitelaw *et al,* 1986) indicate that increased yield is not due to improved efficiency of utilisation of energy from the mobilised fat, but simply increased rates of fat mobilisation. The latter was confirmed in tracer studies with palmitate and acetate during postruminal casein infusions which increased the palmitate entry rates (König *et al,* 1984), although other reports indicate that the efficiency of metabolizable energy (ME) to net energy of lactation (NEl) conversion might be higher when the feed is supplemented with protein (Rulquin, 1982; Chamberlain *et al,* 1989).

It is not clear how absorbed protein elicits the mobilisation of body reserves. One of the most commonly proposed hypotheses is that metabolizable protein could stimulate growth hormone (GH) secretion (Oldham *et al,* 1978; Oldham *et al,* 1982), which, in turn, is responsible for decreased responsiveness by adipose tissue to insulin and increased responsiveness to adrenergic stimuli at the end of gestation and the beginning of lactation. This results in a shift from a fat accretion to a fat mobilisation state. The length of this metabolic adaptation by the adipose tissue is probably influenced by environmental factors (Weeks *et al,* 1983), including diet (Vernon and Sasaki, 1991). The effects of absorbed protein could also be due to changes in the profile of the absorbed amino acids or they could be mediated by gastrointestinal hormones which are known to modulate the activity of the sympathetic nervous system and are released in response to certain amino acids (Choung and Chamberlain, 1992).

Epinephrine challenges have frequently been used to evaluate the responsiveness of adipose tissue to adrenergic stimuli (McCutcheon and Bauman, 1986; Bridges *et al,* 1987; Fröhli and Blum, 1988). The aim of the present experiment was to evaluate whether available dietary protein and the body condition score (CS) at calving, directly or indirectly, affect the responsiveness of the adipose tissue to *in vivo* adrenergic stimulation in dairy cows during early lactation.

MATERIALS AND METHODS

Sixteen multiparous Freisian cows were randomly assigned prepartum to 1 of 2 diets with different energy contents, to achieve different body CS values at calving. Diet A consisted of grass silage *ad libitum* and 2 kg/d of ground barley with the appropriate mineral supplement. Animals on diet B were fed the same grass silage restricted to 80% of the *ad libitum* intake of group A, aiming for a 1.0 unit difference in CS at calving. After calving the animals were group-fed once a day grass silage *ad libitum* and 5 kg/head of concentrate 1 (table I) for the first 2 weeks of lactation and then assigned to 1 of 2 concentrates, differing in the proportion of undegradable intake protein (UIP; NRC, 1989), fed at 5 kg/d until week 14. The protein degradability of the concentrate ingredients and silage was estimated by an inhibitor *in vitro* method (Broderick, 1987) and ruminal escape values calculated assuming a ruminal rate of passage of 0.06 h^{-1}. The animals were subject to an epinephrine challenge immediately after the morning milking at the end of the 3rd week (*ie* when the concentrate treatment had been applied for 7 d) and in the 8th week of lactation. A Teflon iv catheter (Abbocath-T 4535-76) was placed in the jugular vein 2 d before the challenge. A solution of 8 μM epinephrine in saline was infused at a rate of 0.82 nmol/kg body weight/min for 8 min and 5 ml blood was taken at −10, −5, 8, 10, 15, 20, 25, 30, 45 and 60 min relative to the beginning of infusion. Blood was immediately refrigerated at 4°C and the plasma was frozen until analysis for non-esterified fatty acids (NEFA) using a microplate-adapted NEFA-C test (Wako Chemi-

Table I. Concentrate ingredients and composition of concentrate and silage offered to dairy cows during early lactation.

Ingredient (% DM)	*Concentrate*		*Composition*	*Concentrate*		*Silage*
	High UIP	*Low UIP*		*High UIP*	*Low UIP*	
Barley	56.8	56.8	OM (% DM)	93.7	93.2	90.0
Solvent soybean meal	–	40.0	CP (% DM)	24.7	28.6	12.6
Expeller soybean meal	40.0	–	UIP (% CP) [a]	54.0	31.1	29.0
Vitamin–mineral mix	0.2	0.2	ADF (% DM)	8.5	8.7	39.6
Dicalcium phosphate	1.8	1.8	NDF (% DM)	44.5	42.7	57.9
Calcium carbonate	1.2	1.2	NEI (Mcal/kg DM)	1.88	1.88	1.39 [b]

Both concentrates were fed at a fixed rate of 5 kg/head/d; silage *ad libitum*; [a] assuming ruminal fractional rate of passage is 6% h^{-1}; [b] estimated from ADF content.

cals) scaled down as follows. Reagents A and B were prepared as recommended by the manufacturer and then diluted 1:1 with distilled water; a sample volume of 25 µl mixed with 100 µl of diluted reagent A was incubated at 39°C for 20 min and afterwards 200 µl of diluted reagent B was added and incubated for 10 min. Absorbances were read at 550 nm. Basal and average concentrations and the average and maximum increases in NEFA concentrations were determined. Plasma concentrations were plotted against time and the area under the curve was calculated.

Statistics

Analysis of variance was carried out using the GLM procedure of SAS. The overall random effects of week of lactation, absorbable protein in the diet and CS group at calving and their interactions were tested using the model:

$$Y_{ijk} = \mu + W_i + C_j + P_k + (C \times P)_{jk} + (W \times C)_{ij} + (W \times P)_{ik} + (W \times C \times P)_{ijk} + e_{ijk}$$

where Y_{ijk} = the dependent variable; μ = the population mean; W_i = the effect of the ith week of lactation; C_j = the effect of the jth condition score group; P_k = the effect of the kth level of absorbable protein and e_{ijk} = the error associated with the ijkth observation, assumed to be identically and independently distributed as a normal distribution $(0, \sigma^2_e)$.

A significant effect of week on basal concentrations of NEFAs was observed and so the data set for each week was analysed independently with the model:

$$Y_{jk} = \mu + C_j + P_k + (C \times P)_{jk} + e_{jk}$$

RESULTS

The average CS value and the range of CS values at calving were 4.56 ± 0.21 (5–3.5) and 3.75 ± 0.27 (4.5–2.5) for the high and low CS groups respectively (on a 0 to 8 scale, Earle, 1976). The grain fed differed only in the protein concentrate which was solvent-extracted soybean meal (SBM) for concentrate 1 and expeller-extracted SBM for concentrate 2. Protein degradability was 35% (crude protein (CP) basis) for the solvent SBM and 57% for the expeller SBM. Lactation performance, body weight and the CS of the animals in the days preceding the challenges are given in table II. All animals lost body weight from calving to week 3 and then gained weight up to week 8.

Table II. Average dry matter intake over the experimental period, milk yields, body weights and condition scores (CS) at the time of the epinephrine challenges and before the beginning of the experiment of cows calving with 2 different CS and fed concentrates differing in their undegradable protein (UIP) from the 3rd to 8th week of lactation.

	High CS at calving		*Low CS at calving*	
	High UIP	*Low UIP*	*High UIP*	*Low UIP*
Dry matter intake (kg/d)	15.3	15.6	15.6	14.9
Milk yield (kg/d)				
Week 2	24.2 ± 1.0	23.8 ± 1.5	21.5 ± 1.7	21.1 ± 1.2
Week 3	25.8 ± 1.8	23.7 ± 1.3	22.4 ± 1.6	22.5 ± 2.0
Week 8	25.0 ± 0.9	23.5 ± 2.2	23.1 ± 2.6	21.6 ± 1.2
Live weight (kg)				
Calving	528 ± 27	511 ± 30	476 ± 25	482 ± 39
Week 3	510 ± 29	497 ± 40	466 ± 23	475 ± 11
Week 8	514 ± 28	510 ± 26	487 ± 32	497 ± 13
Condition score				
Calving	4.75 ± 0.14	4.38 ± 0.38	3.62 ± 0.43	3.83 ± 0.44
Week 3	4.63 ± 0.36	4.00 ± 0.53	3.63 ± 0.28	3.88 ± 0.14
Week 8	4.13 ± 0.36	4.88 ± 0.49	3.88 ± 0.14	4.13 ± 0.28

The basal concentration of NEFA was higher in animals from the high CS group and in those fed the high UIP diet (table III). There was a significant interaction between the CS and the dietary UIP level, with a very pronounced effect of UIP on basal NEFA concentrations in the animals that calved with a high CS, but not in those calving with a low CS. In fact, the basal concentration in low CS animals fed the high UIP diet was not different from that of animals fed the low UIP ration (regardless of their CS group). Basal concentrations of NEFA in week 3 were higher than in week 8 of lactation (0.27 *vs* 0.15 mEq l^{-1}; $P < 0.01$). There were no interactions between week of lactation and CS or UIP group.

After the epinephrine challenge, plasma NEFA reached concentrations of 0.56 mEq l^{-1} in cows on the high UIP diet while in the low UIP group concentrations remained below 0.34 mEq l^{-1}. Again, the UIP level and the CS interacted positively: the maximum NEFA concentrations reached 0.74 mEq l^{-1} in the high UIP/high CS group while maximum concentrations in the other 3 groups ranged from 0.32 to 0.37 mEq l^{-1}. The maximum concentrations reached after the challenge were not significantly different between weeks 3 and 8 of lactation. When the increase in NEFA concentrations after the challenge was adjusted by subtracting the basal concentrations, the maximum increase in NEFA concentration was larger ($P < 0.056$) for the high UIP group, but the effects of CS or its interaction with UIP level disappeared (table III). The area under the curve (fig 1) was also significantly larger in the animals fed the high UIP diet and in cows that calved with a high CS than in the low UIP and low CS groups, respectively. There was no interaction between UIP and CS. When the data from weeks 3 and 8 were analysed

Table III. Plasmatic NEFA concentrations (mEq l^{-1}) and area under curve after epinephrine challenge (mEq l^{-1} min) in cows calving with 2 different condition scores (CS) and fed 2 different levels of undegradable protein (UIP) postpartum.

	High CS at calving		*Low CS at calving*		*Effects (p)*		
	High UIP	*Low UIP*	*High UIP*	*Low UIP*	*CS*	*UIP*	*CS•UIP*
Basal [a,b]	0.39	0.13	0.18	0.15	0.030	0.003	0.01
Week 3	0.50	0.13	0.24	0.22	0.270	0.016	0.03
Week 8	0.28	0.14	0.11	0.08	0.029	0.090	0.23
Maximum [b]	0.74	0.35	0.38	0.32	0.026	0.012	0.05
Week 3	0.82	0.29	0.51	0.41	0.390	0.013	0.06
Week 8	0.66	0.41	0.25	0.23	0.042	0.309	0.37
Maximum increment [b]	0.33	0.20	0.27	0.17	0.40	0.056	0.78
Week 3	0.33	0.16	0.26	0.20	0.829	0.106	0.47
Week 8	0.33	0.24	0.28	0.15	0.406	0.248	0.83
Area under curve [b]	6.47	3.31	3.77	2.48	0.054	0.018	0.29
Week 3	5.16	2.53	3.19	2.72	0.481	0.230	0.39
Week 8	7.78	4.09	4.34	2.24	0.010	0.011	0.20

[a] Week effect, $P < 0.01$; [b] average of weeks 3 and 8.

separately we observed that the effect of UIP level on the tissue response to epinephrine challenge was significant in week 8 but not in week 3. However, the analysis of the complete data set did not reveal significant week effects or interactions between week of lactation and UIP effects on tissue response to epinephrine.

DISCUSSION

The 0.8 unit difference in the condition score at calving, although less than expected, was large enough to produce significant differences in milk yield. The range of CS for each group overlapped and it was felt that the observed effects could be attributed more to the dry period feeding than to the actual calving CS. However, the effect of CS group on the variables measured became non-significant when the effect of the actual CS measured at the time of calving was accounted for in the model, indicating that the observed effects of CS group were related to the actual CS of the animals at calving.

The dietary treatment effect on adipose tissue function was already apparent by week 3, as indicated by the significant differences in basal NEFA concentrations observed. The basal plasma concentrations of NEFA correlated well with the rates of fat mobilisation, as shown by Bauman *et al* (1988). Our basal NEFA values indicated that fat mobilisation was more intense in the high CS animals that received the high UIP diet than in any of the other groups. The UIP effect on basal NEFA concentrations was in agreement with higher rates of adipose mobilisation reported in dairy animals supplemented with postruminally infused

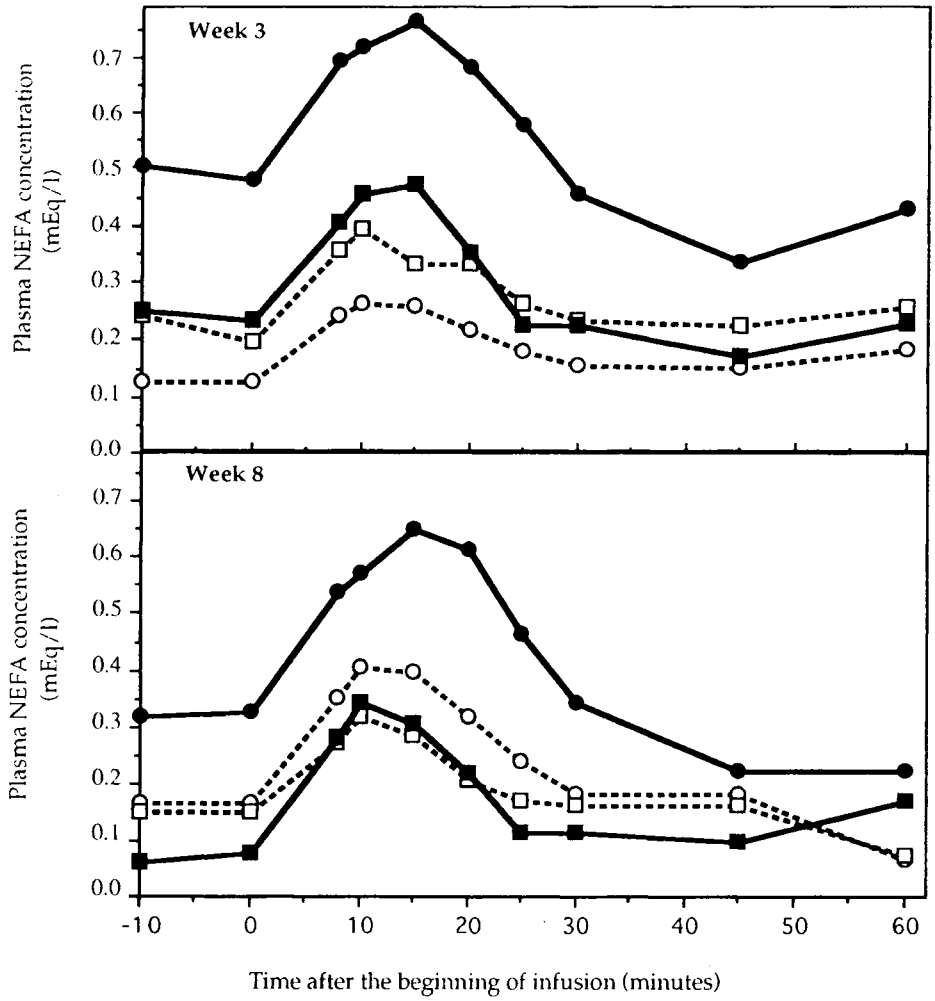

Fig 1. Plasmatic NEFA concentrations (mEq l^{-1}) after an epinephrine challenge (0.82 nmol/kg body weight/min for 8 min) on weeks 3 and 8 of lactation in cows that calved with high (O,●) or low (□,■) condition scores, fed diets with high (●,■) or low (O,□) undegradable protein content.

protein (Ørskov *et al,* 1977). The significant interaction between CS and UIP level was also in accordance with the results of Garnsworthy and Jones (1987) who found that the addition of undegradable protein to the diet increases the rate of body weight loss in well-conditioned animals, but not in those calved with a low condition score.

Animals fed the high UIP diet responded to the epinephrine challenge by mobilising a larger amount of fat (higher area under the curve) and reached higher increments of NEFA concentrations, even after adjusting for their already larger basal levels. This significant effect of UIP on the *in vivo* response to epinephrine indicated that

nutritional factors, absorbable protein in this case, did modulate the endocrine adaptations of lactation. Greater *in vivo* responsiveness to epinephrine is usually present with enhanced milk energy yield, a common response to high UIP diets also seen in this experiment. The effects of CS group could be related to differences in the size of the pool of mobilisable fat, but also to probable differences in adipocyte numbers and sizes, which were usually associated with differences in CS and could have differential responsiveness to catecholamines (Smith and McNamara, 1990).

The fact that the effect of UIP level on tissue response to epinephrine challenge was significant on week 8 but not on week 3 is difficult to interpret. McNamara (1988) found that, relative to prepartum values, *in vitro* NEFA release after epinephrine stimulation was increased on day 30 postpartum and remained elevated until 240 d into lactation. In a previous work (McNamara and Hillers, 1986), the response to epinephrine had its maximum at day 30 and then dropped to values slightly above prepartum on day 60 of lactation. Our results showed a non-significant rise in adipose tissue responsiveness to epinephrine between weeks 3 and 8. If the results of McNamara and Hillers (1986) are correct, our data would suggest that UIP treatment tends to delay the drop in tissue responsiveness to epinephrine as lactation proceeds. On the contrary, if adipose responsiveness to adrenergic stimulation remains stable through late lactation (McNamara, 1988), UIP addition to the diet might act to prolong the period over which responsiveness rises after parturition. This cannot be established with our experiment since measurements were taken only at 2 time points in lactation. All animals lost body weight during week 3 and gained weight in week 8, therefore the differences in responsiveness within each period can not be attributed to major differences in energy balance, although cows on the high UIP treatment stayed on a negative energy balance longer than their controls. The latter might be important if once the cows start regaining body weight there is a lag time in which the increased responsiveness to epinephrine is still present.

In conclusion, our results confirm that available protein has an impact on the adipose tissue's ability to mobilise fat. They also suggest that this could be achieved by maintaining an increased responsivenesss of the tissue to epinephrine for a longer period after the beginning of lactation.

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