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PROCEEDINGS OF THE NUTRITION SOCIETY

ABSTRACTS OF COMMUNICATIONS

The Four Hundred and Forty-eighth Meeting of the Nutrition Society (One Hundred and Seventy-seventh of the Scottish Group) was held in the Craigie College of Education, Ayr on Thursday and Friday, 7/8 April 1988, when the following papers were read:

https://doi.org/10.1079/PNS19880062 Published online by Cambridge University Press

The effect of practice on the measurements of skinfold thickness by an inexperienced observer. By J. WALKER and S. KINDLEN, *Department of Science and Dietetics, Queen Margaret College, Edinburgh*

Skinfold thickness measurement is often employed in the assessment of body fat. The technique is inexpensive, non-invasive and apparently easy, but it is open to a number of assumptions and possibilities of error. The present study examined the effect of practice following training on the results obtained by a previously inexperienced observer.

Eighteen subjects were measured, all were female, aged 20–25 years and with fat mass ranging from 18 to 32% of total body mass. The same Harpenden caliper was used throughout and measurements were made according to the method of Durnin & Womersley (1974) at the biceps, triceps, suprailiac and subscapular sites. After a period of instruction and preliminary practice to eliminate obvious errors in technique, the inexperienced observer made five sets of measurements over a period of 14 d. The results of these measurements were subsequently compared with those made by an experienced observer at the time of the first measurements by the inexperienced observer.

Mean percentage difference between skinfold thickness measurements made on eighteen subjects by an inexperienced and experienced observer

Successive sets of measurements	Biceps		Triceps		Subscapular		Suprailiac		Total	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
1	−19·4	15·9	−21·5	14·1	−0·7	18·3	−26·6	10·9	−18·2	8·4
2	−8·1	17·7	−13·2	16·3	−0·6	12·5	−19·9	14·4	−12·1	10·4
3	−3·5	9·6	−4·9	6·1	+1·1	5·5	−10·8	11·3	−4·6	4·8
4	+1·8	6·7	−4·4	5·7	+1·6	3·7	−3·9	6·4	−2·0	3·3
5	+1·2	5·2	−4·8	4·1	+1·9	3·7	−3·0	6·3	−1·8	2·8

At the final measurement, there was no significant difference, using a paired t test at the 0·01 level, at the biceps (P=0·469), subscapular (P=0·025) and suprailiac (P=0·041) sites, but there was still a significant difference at the triceps site (P<0·0001) and in the measurement totals (P=0·0081). When the results were converted to percentage body fat (Durnin & Womersley, 1974) the differences between the mean body fat percentage for the eighteen subjects as estimated by the experienced observer and the inexperienced observer on five occasions were 2·9, 1·9, 0·8, 0·3 and 0·3% respectively. The difference at the first measurement was highly significant (P<0·0001); however, at the last measurement, the difference was no longer significant (P>0·01).

The results indicate that significant error in body-fat estimation by skinfold thickness measurement may be introduced by using an inexperienced observer but that this error may be reduced to an insignificant level by a relatively short period of practice.

Durnin, J. V. G. A. & Womersley, J. (1974). *British Journal of Nutrition* **32**, 77–97.

Unidirectional calcium and phosphate fluxes across the isolated mucosa from the rumen and omasum of sheep. By G. BREVES* and H. HÖLLER, *Department of Physiology, School of Veterinary Medicine, Hannover, FRG*

There have been few studies on the function of the rumen and the omasum as sites of inorganic phosphate (P_i) and calcium absorption in sheep. Recent in vivo experiments using the washed and temporarily isolated reticulorumen of sheep have, however, shown that the reticulorumen substantially contributes to Ca and P_i absorption in sheep (Breves *et al.* 1988; H. Höller, G. Breves, M. Kocabatmaz and H. Gerdes, unpublished results).

In order to characterize the transport mechanisms involved, unidirectional P_i and Ca flux rates across isolated mucosal discs from the rumen and the omasum were measured in vitro using Ussing chambers. Within 2–3 min after slaughter, pieces from the ventral sac of the rumen and omasal leaves were isolated and cleaned with isotonic buffer solution. The mucosa was stripped from the muscle layer and then mounted between the two halves of Ussing chambers. Identical buffer solutions were given to both sides of the tissue, kept at 39° and continuously gassed, using a gas lift system, with humidified oxygen:carbon dioxide (95:5). When the electrical characteristics of the tissues (potential difference, conductance, short-circuit current) showed differences of less than 20%, these tissues were paired, and ^{32}P as orthophosphate or ^{45}Ca as $CaCl_2$ were added either to the serosal or to the mucosal side of the tissue for calculation of unidirectional flux rates. Three flux periods of 30 min each were run in each chamber under short-circuit current conditions.

Neither in ruminal nor in omasal tissues did P_i net fluxes occur in the absence of an electrochemical gradient, indicating that P_i absorption in both organs is by passive diffusion, and hence dependent on the existing electrochemical gradient. There was a significant Ca net flux from the mucosal to the serosal side in both tissues. When Na^+,K^+-ATPase was inhibited by adding ouabain to the serosal side, the Ca net flux was abolished. Similar results were obtained with sodium-free buffer solutions. These results demonstrate that Ca is absorbed actively from the rumen and the omasum and that transport depends on an intact Na^+,K^+-ATPase and the presence of sodium ions.

Breves, G., Höller, H., Packheiser, P., Gaebel, G. & Martens, H. (1988). *Quarterly Journal of Experimental Physiology* (In the Press).

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Glucose feeding during starvation and its effects on secretion in the rat ileum and colon.

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Giving rats an isotonic glucose solution during starvation ameliorates the hypersecretory state associated with starvation in the jejunum (Young & Levin, 1987). We have now investigated the effects of giving a glucose drink (55 g/l) during a 72 h starvation period on secretion in the rat terminal ileum and proximal colon. Four groups of rats were used: fed controls, 72 h fasted, 72 h fasted with the glucose solution available *ad lib.*, and 72 h fasted with saccharin-sweetened water available *ad lib*. Saccharin was used to encourage the rats to drink water. Electrogenic ion secretion was monitored as the short-circuit current (Isc) using standard methods. Basal Isc and maximal increases in Isc above basal levels (ΔIsc) in response to 1 mM-Bethanecol at the serosa were measured. The results are shown in the Table.

Basal Isc and ΔIsc in ileum and colon (μA/cm² serosal area)

		Ileum				Colon			
		Basal Isc		ΔIsc		Basal Isc		ΔIsc	
	n	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Fed	33	46	3	51	6	72	3	58	5
Fasted 72 h	36	79	6	127	10	57	3	87	5
Fasted + saccharin	20	77	4	119	8	53	4	85	10
Fasted + glucose	20	60	7	71	7	56	3	88	6

Statistical analysis used the Kruskal–Wallis (non-parametric ANOVA) followed by Connover's multiple comparison test.

Giving glucose during starvation partly ameliorated the hypersecretion associated with fasting in the ileum, lowering the basal Isc by 24% ($P<0.05$) and the ΔIsc by 79% ($P<0.01$) compared with the 72 h fasted group. However, the basal Isc (+30%; $P<0.05$) and ΔIsc (+39%; $P<0.05$) of the glucose-fed rats were still elevated compared with fed controls. Saccharin in the drinking water did not change the effects of fasting. In the proximal colon glucose feeding and saccharin feeding during starvation had no effect, the basal Isc being depressed and the ΔIsc being elevated as in the 72 h fasted rats.

Thus glucose feeding ameliorates the starvation-induced hypersecretion in the rat jejunum (Young & Levin, 1987), partly ameliorates it in the ileum but has no effect in the proximal colon.

Financial support from the British Digestive Foundation is gratefully acknowledged.

Young, A. & Levin, R. J. (1987). *Proceedings of the Nutrition Society* **46**, 24A.

Inverse relation between DNA synthesis and casein mRNA synthesis in mammary epithelial organoids from mid-pregnant mice. By M. C. BARBER and R. R. DILS, *Department of Physiology and Biochemistry, University of Reading, PO Box 228, Whiteknights, Reading RG6 2AJ*

The ability of mouse mammary tissue to incorporate tritiated thymidine into DNA (a measure of cell proliferation) is high during pregnancy and falls to low levels during lactation (Knight & Peaker, 1982). Cultures of lactating-mouse mammary cells can be stimulated to proliferate with tumour-promoting phorbol esters, although this occurs at the expense of the ability to synthesize casein (Taketani & Oka, 1983). In the present study, mammary organoids obtained by collagenase digestion of mid-pregnant mouse mammary tissue were cultured for up to 96 h in hormone-supplemented culture medium on a matrix prepared from rat-tail collagen. At appropriate times in culture (Table), the DNA synthetic capacity of the organoids was measured by the incorporation of tritiated thymidine and RNA was isolated to estimate α-casein mRNA by hybridization with a specific mouse α-casein cDNA probe.

Period in culture (h)	Hormone combination	DNA content (μg/culture)	DNA synthesis (dpm/μg DNA per 4 h)	Relative casein mRNA synthesis†
4	No hormones	—	1400[a]	3
24	IC	16·3	3200[b]	1
24	ICP	12·2	6200[c]	3
96	IC	13·0	900[a]	1
96	ICP	12·2	700[a]	10
n		4	4	3
SE of difference		1·2	1300	—

I, insulin (0·85 μmol/l); C, cortisol (2·5 μmol/l); P, prolactin (4·5 nmol/l); *n*, number of independent experiments; dpm, disintegrations/min.

[a,b,c] Values in a column with different superscript letters differed significantly ($P<0·01$).

†Expressed as relative hybridization intensity.

Tissue culture for 24 h resulted in a marked increase in the rate of DNA synthesis by the mammary organoids, the rate being significantly greater in ICP than IC medium. By contrast there were no differences in tissue DNA contents (a measure of the number of cells) in IC or in ICP medium during the 96 h culture. After 24 h culture the level of α-casein mRNA was maintained in ICP medium, whereas culture in IC medium produced a reduction. After 96 h culture in ICP medium, there was a striking increase in α-casein mRNA which coincided with a marked decrease in the rate of DNA synthesis (Table). The inverse relation between DNA synthesis and casein mRNA synthesis supports the view that proliferation and differentiation in these cells may be mutually exclusive.

Knight, C. H. & Peaker, M. (1982). *Quarterly Journal of Experimental Physiology* **67**, 165–177.
Taketani, Y. & Oka, T. (1983). *Proceedings of the National Academy of Sciences, USA* **80**, 1646–1649.

Zinc, copper and manganese in human milk in late lactation. By C. E. CASEY, *Department of Medicine and Therapeutics, University of Aberdeen, Aberdeen AB9 2ZD*

Accurate information on the concentrations of trace elements in human milk, and their relation to volume and other milk constituents throughout lactation, is important both for formulating nutritional requirements and to provide a baseline for understanding the physiology of their secretion. Information on volume of milk produced, milk consumption, maternal diet, and infant growth and diet were obtained at monthly intervals from thirteen healthy multi-parous women in Denver, Colorado, as part of a large study of lactation performance. Results reported here include results from 5 months post-partum to weaning (8–17 months) or to the end of the study (10–19 months) in women who had not weaned.

The Table gives mean (SD) values at selected months for concentration of zinc, copper and manganese (with number of subjects) and milk intake by the infant. Zn and Cu include values from all subjects; because Mn levels were affected by weaning, subjects were only included in the mean while they were producing >399 g milk/d. Milk intakes include only those of infants receiving <418 kJ (<100 kcal)/d.

Period post-partum (months)	Zn (μmol/l)			Cu (μmol/l)			Mn (nmol/l)			Milk intake (g/d)		
	Mean	SD	*n*	Mean	SD	*n*	Mean	SD	*n*	Mean	SD	*n*
5	18·5	7·9	13	3·5	0·8	13	41	20	13	838	134	12
7	14·1	7·0	13	2·6	1·0	13	40	24	13	848	63	6
9	11·8	6·0	11	2·8	0·9	11	42	19	11	817	91	4
11	8·1	5·0	11	2·4	0·8	11	63	23	8	649	93	8
13	4·8	4·1	5	2·6	0·7	4	69	—	1	418	—	1
15	4·5	2·6	6	3·5	0·8	3	84	—	1	759	—	1

A triple regression technique ((a) volume *v.* days post-partum; (b) trace element concentration *v.* days; (c) slopes from (a) *v.* slopes from (b) for each subject) was used to distinguish between decline in trace element concentrations due to period post-partum and that due to decline in milk volume with gradual weaning. From 5 to 13 months, concentrations of Zn declined significantly with time ($P<0·01$) but were not affected by declining volumes. Cu levels were not changed with time or volume between 6 and 13 months. Mn concentrations increased with time over 6–13 months ($P<0·05$); there was no effect of milk volume in women producing >399 g milk/d. Below this volume, large, irregular changes were observed in Mn levels.

Supported by contract no. 01 HD 22801 and grant nos. HD-19547 and 5R22 AM12432 from the National Institute of Health, and grant no. RR-69 from the General Clinical Research Centers.

Interactions between breast and supplementary feeding in rural northern Thailand. By S. M. IMONG[1], D. A. JACKSON[1], A. SILPRASERT[2], L. WONGSAWASDII[2], P. CHIOWANICH[2], M. W. WOOLRIDGE[1], J. D. BAUM[1] and K. AMATAYAKUL[2], [1]*The Institute of Child Health, Bristol University, Bristol BS2 8BJ* and [2]*Research Institute for Health Sciences, Chiang Mai University, PO Box 80 CMU, Chiang Mai 50002, Thailand*

The interaction between breast-feeding and supplementary feeding was examined in a random sample of forty-four mixed-fed infants, aged between 0 and 12 months, from rural northern Thailand, studied in their homes over 48 h. All daytime (awake hours) feeds of breast milk, supplements and water were measured by test weighing using electronic averaging balances (resolution 1 g). Night-time breast-milk intake was measured using indirect test weighing (Woolridge *et al.* 1987). Expressed breast-milk samples (0·5 ml) were taken before and after each daytime feed for analysis of fat content. Feed supplements were sampled for measurement of energy content. Results for seven exclusively breast-fed infants are available for comparison.

For all subjects, mean 24 h breast-milk intake was 579 (SD 187) g, range 125–983 g, but in the seven exclusively breast-fed infants was 696 (SD 100) g, range 575–826 g. Up to 6 months of age, 53% of supplementary feeds were rice-based, generally accompanied by banana, and small amounts of meat, egg or fish thereafter. Other foods given included commercial cereals (8%), snacks (14%), fruit (17%) infant formula (6%). Mean 24 h supplementary food intake was 143 (SD 144) g, range 0–777 g. Breast feeds provided a median of 150 kJ (36 kcal) per feed, supplementary feeds 159 kJ (38 kcal).

Mean 24 h breast-milk intakes and supplementary food intakes were negatively associated (r −0·453, $P<0·01$), as were mean 24 h breast and supplementary feed frequencies (r −0·359, $P<0·01$). There was no association between total food intake (breast and supplementary feeding combined) and infant age, expressed either as g or as kJ. Total food intake thus remained approximately constant over the whole of the first year of life. Analysis of feed spacing showed that supplementary feeds probably replaced breast feeds, with more time between breast feeds than in exclusively breast-fed infants (a mean of 165 and 116 min apart respectively). The negative association between breast and supplementary feeding seemed to come about through a reduction in breast-feeding episodes when supplements were given, rather than through the amount of breast milk consumed at a feed. Both breast and supplementary feeds were scheduled (that is, they tended to fall at similar times on both study days) and it is suggested that schedules may limit the number of feeding episodes available to the infant, thus restricting total nutrient intake.

The debate on the appropriate timing for introducing supplements, which assumes that supplements provide extra energy to the diet as breast milk becomes inadequate for growth, may be misleading if, in practice, supplements replace rather than truly 'supplement' breast-milk intake.

Woolridge, M. W., Jackson, D. A., Imong, S. M., Yootabur, Y. & Amatayakul, K. (1987). *Human Nutrition: Clinical Nutrition* **41C**, 347–361.

Effects of tumour implantation on the onset of lactation in the rat. By R. D. EVANS and D. H. WILLIAMSON, *Metabolic Research Laboratory, Nuffield Department of Clinical Medicine, Radcliffe Infirmary, Woodstock Road, Oxford OX2 6HE*

A model has been developed in which the Walker 256 carcinosarcoma is implanted subcutaneously in the lactating rat in order to delineate possible interactions between the metabolism of the tumour and the mammary gland (Evans & Williamson, 1988). Previous observations demonstrated that lactational performance (pup weight gain) and mammary gland metabolism (lipogenesis, lipoprotein lipase (*EC* 3.1.1.34) activity) is unaffected by tumour burden up to 3–5% of body mass if the tumour is implanted post-parturition. In the present study the effects of tumour implantation before parturition on the development of lactation immediately after birth was examined.

Wistar rats (200–250 g) were inoculated with 2×10^7 cells of Walker 256 carcinosarcoma or saline (9 g sodium chloride/l) 1–3 d prepartum. Food intake and pup weight were measured daily. The rate of mammary gland lipogenesis was measured by incorporation of ^3H_2O into saponified lipids (1–3 d) post-partum and a sample of the mammary gland removed for determination of lipoprotein lipase activity.

		Maternal				Mammary gland				Pups	
		Food intake/ last 24 h (g)		Weight of GIT (g/kg body-wt)		Lipogenesis (μmol ^3H_2O/g tissue per h)		LPL activity (nmol FA released/min per mg acetone dried tissue)		Mass change/ pup last 24 h (g)	
	n	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
Lactating control	4	28·3	4·8	56·7	5·9	49·9	10·9	1·32	0·04	0·70	0·16
Lactating tumour-bearing	6	10·5*	3·1	36·4*	3·3	15·7*	6·7	0·69**	0·11	0·15*	0·17

Significantly different from control: $^*P<0·05$, $^{**}P<0·01$.
GIT, gastrointestinal tract; LPL, lipoprotein lipase; FA, fatty acids.

Despite tumour size being less than 1 g (<0·5% body mass), at this stage (2–4 d of tumour growth) food intake was depressed; an effect not seen at this level of tumour burden in virgin or established lactating rats. The weight of the gastrointestinal tract was lower in the tumour-bearing rats indicating a failure of the expected hypertrophy to occur. Despite similar litter weights at birth, the pups of the tumour-bearing rats gained less weight than the control pups. Evidence for altered metabolism in the lactating gland was a decreased rate of lipogenesis and of lipoprotein lipase activity. The former may be due to the relative hypophagia (Mercer & Williamson, 1987).

These results indicate that the presence of a small tumour, insufficient to affect established lactation, can interfere with a number of physiological and metabolic processes associated with the onset of lactation in the rat.

Evans, R. D. & Williamson, D. H. (1988). *Biochemical Journal* (In the Press).
Mercer, S. W. & Williamson, D. H. (1987). *Biochemical Journal* **242**, 235–243.

Inhibition of insulin release by mannoheptulose decreases mammary gland glucose uptake, lipogenesis and phosphofructokinase activity in starved–refed lactating rats.

By S. W. MERCER and D. H. WILLIAMSON, *Metabolic Research Laboratory, Nuffield Department of Clinical Medicine, Radcliffe Infirmary, Woodstock Road, Oxford OX2 6HE*

Refeeding a chow diet to starved, lactating rats leads to a rapid rise (0–20 min) in plasma insulin, and a later increase (30–90 min) in lipogenesis in the mammary gland, by which time plasma insulin concentrations have returned to a low level (Mercer & Williamson, 1986; Page & Kuhn, 1986). Insulin plays an important role in mammary gland lipogenesis, but the precise metabolic control of glucose metabolism by insulin is not fully understood. Recent work suggests that the rapid changes in lipogenesis in the gland of lactating rats during starvation–refeeding involve the modulation of phosphofructokinase (PFK) activity (Mercer & Williamson, 1987). The aim of the present study was to investigate the influence of inhibition of insulin secretion (with mannoheptulose) before and after the peak insulin response, on mammary gland glucose uptake, lipogenesis and PFK activity during refeeding of starved, lactating rats.

Lactating Wistar rats (11–14 d lactation) were fasted for 18 h. Refed rats received 5 g chow diet which they ate within 20 min. Insulin secretion was blocked by intraperitoneal injection of mannoheptulose (500 mg) either 30 min before refeeding or 30 min after the onset of refeeding. Lipogenesis was measured in vivo with ^3H_2O, and glucose uptake was assessed by arteriovenous difference across the gland.

Refeeding led to a significant increase in glucose uptake by the gland by 30 min, associated with a decrease in gland glucose-6-phosphate and an increase in fructose 1,6–bisphosphate concentrations, indicative of an activation of PFK. Mannoheptulose, given before refeeding, completely abolished these changes in glucose uptake and PFK activity at the 30 min time point.

Refeeding was also associated with a large (twentyfold) increase in lipogenesis in the mammary gland, over the 30–90 min post-refeeding period, and this was also associated with increased glucose uptake and PFK activity (determined at 90 min). However, administration of mannoheptulose at 30 min post-refeeding, prevented this activation of lipogenesis in the gland, and the increase in glucose uptake and PFK activity. Thus, the increase in glucose uptake and PFK activity observed by 30 min of refeeding, can be rapidly reversed by inhibition of insulin secretion (by mannoheptulose) over the 30–90 min period, despite the fact that the plasma insulin has already returned to a low level by this time (Mercer & Williamson, 1986). These results suggest that the insulin sensitivity of the mammary gland of the lactating rat may be acutely enhanced by refeeding, and that insulin-stimulation of PFK may be important in the regulation of lipogenesis in the tissue.

Mercer, S. W. & Williamson, D. H. (1986). *Biochemical Journal* **239**, 489–492.
Mercer, S. W. & Williamson, D. H. (1987). *Biochemical Journal* **242**, 235–243.
Page, T. & Kuhn, N. J. (1986). *Biochemical Journal* **239**, 269–274.

Effect of feeding level on insulin sensitivity during lactation in the ewe. By J. A. METCALF* and T. E. C. WEEKES, *Department of Agricultural Biochemistry and Nutrition, University of Newcastle upon Tyne, Newcastle upon Tyne NE1 7RU*

Insulin is a major determinant of nutrient partitioning during lactation. Glucose uptake by the ruminant mammary gland is not responsive to insulin, but by stimulating uptake by other tissues, the hormone reduces glucose availability for milk synthesis.

We examined whole body insulin sensitivity using the euglycaemic insulin clamp technique (Janes *et al.* 1985) in ewes fed on a concentrate diet either *ad lib.* or at a restricted level which resulted in a loss of body-weight throughout lactation. Lambs were weaned at 70 d post-partum. Insulin sensitivity is expressed as the plasma insulin concentration (ED_{50}) resulting in half-maximal glucose metabolic clearance rate (MCR). Values were obtained as ln ED_{50} by plotting MCR *v.* ln (plasma insulin concentration).

Restricted intake groups showed a high ED_{50} which indicated a decreased insulin sensitivity throughout the experiment. *Ad lib.* intake groups showed no consistent pattern in ED_{50} (Table).

ED_{50} values during and after lactation

	Restricted intake			*Ad lib.* intake			
Period post-partum (d)	27	63	105	29	44	64	99
Ln ED_{50}: Mean	5·55	5·56	5·14	4·85	4·53	5·13	5·29
SEM	1·07	1·08	1·07	1·05	1·05	1·05	1·05
ED_{50} (μU/ml)	257	260	171	128	93	170	198
No. of animals	5	6	5	8	8	8	7

ED_{50} did not change significantly over lactation, but ED_{50} values were higher ($P<0·05$) for restricted-intake ewes compared with animals fed *ad lib.*

The ED_{50} values for restricted-intake ewes tended to be higher in early lactation whereas sensitivity of *ad lib.*-intake ewes decreased after peak lactation when the animals were increasing in body-weight. ED_{50} values were higher than previously reported (Brockman, 1983; Janes *et al.* 1985) in non-breeding sheep, especially when intake was restricted.

It would appear that a major determinant of insulin sensitivity during lactation is the feeding level, although there are homeorhetic changes in response to lactation.

This work was supported by Pfizer (UK) Ltd and the SERC.

Brockman, R. P. (1983). *Comparative Biochemistry and Physiology* **74A**, 681–685.
Janes, A. N., Weekes, T. E. C. and Armstrong, D. G. (1985). *British Journal of Nutrition* **54**, 459–471.

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Changes in circulating insulin and in insulin-receptor affinity of erythrocytes in dairy cows around parturition. By M. STANGASSINGER and D. GIESECKE, *Department of Animal Physiology, University of Munich, Munich, West Germany*

In lactating cows, low circulating levels as well as sluggish responses of insulin to both physiological (feeding) and artificial stimuli (a glucose load) are considered to be concerned with the diversion of nutrients away from tissue deposition toward milk synthesis.

Since hormonal control is a function of several processes, including blood concentration as well as the number and affinity of hormone receptors at the target tissues, an insulin receptor assay (IRA) for erythrocytes (Gambhir *et al.* 1977) was applied to Holstein×Friesian cows (100 d milk yield: 2987 (SE 267) kg fat-corrected milk, *n* 7) pre- and post-partum. Preliminary investigations with fed and fasted goats (Beck *et al.* 1983) showed that estimates of the tissue sensitivity to insulin provided by the euglycaemic clamp test and the IRA with erythrocytes were significantly correlated.

The status of insulin sensitivity (given as the half-saturation constant (HSC) of the specific insulin-receptor binding to erythrocytes) and the circulation levels of insulin in dairy cows from 9 weeks prepartum to 12 weeks post-partum are given in the Table.

Plasma insulin and insulin-receptor binding to erythrocytes in dairy cows

Period of experiment (weeks)	Insulin (mU/l)			HSC* (mU/assay)		
	Mean	SD	*n*	Mean	SD	*n*
Prepartum						
9	13·2[a,d]	5·2	7	1·26	0·45	5
6	10·8	4·5	7	1·41[a]	0·19	6
3	9·7[b,d]	3·0	7	2·95	1·49	6
Post-partum						
3	3·8[b,c]	1·9	7	3·30	1·47	7
6	6·5	4·0	6	3·78[b]	1·77	6
9	10·7	5·5	6	2·36	2·11	6
12	9·8[a]	1·8	6	1·58[a]	0·75	6

Mean values within vertical columns with different superscript letters were significantly different: [a–d]$P<0·01$ for insulin; [a,b]$P<0·05$ for HSC.

The significant 70% decrease in circulating insulin from 6 weeks prepartum to 3 weeks post-partum and the significant 2·7-fold increase of HSC from 6 weeks prepartum to 6 weeks post-partum indicated a severe insulin deficiency and a marked loss in insulin-binding affinity in early lactation. Interestingly, analogous to the decrease in circulating insulin, the decrease in receptor affinity was initiated several weeks prepartum.

The observations suggest that the fulfilment of the mammary demands at least in early lactation is guaranteed by two very effective mechanisms: insulin deficiency and insulin resistance.

Beck, U., Stangassinger, M., Giesecke, D. & Meyer, J. (1983). In *Proceedings of the 5th International Conference on Production Disease in Farm Animals*, p. 175. Uppsala: Swedish University of Agricultural Science.

Gambhir, K., Archer, J. A. & Carter, L. (1977). *Clinical Chemistry* **23**, 1590.

The use of ammonium acetate for milk-fat production in high-producing dairy cows. By J. N. SWART, P. I. WILKE, H. J. VAN DER MERWE and A. SMITH, *Department of Animal Sciences, University of the Orange Free State, Bloemfontein, South Africa*

Observations by Baldwin *et al.* (1985) suggest that within a physiological state and diet, patterns of nutrient uptake and oxidation by tissues are dependent on concentrations of metabolites in the blood, rates of ATP use in the tissue, and affinity of the tissue for nutrients. Acetate is the major lipogenic precursor in the ruminant mammary gland (Moore & Christie, 1981). Cows fed on high-grain, low-fibre diets showed a reduced secretion of milk fat (Bell, 1981). This appeared to be primarily due to a decreased concentration and mammary uptake of blood acetate (Bell, 1981) originating from a decrease in the molar proportion of acetate in the rumen. Although alternative theories on the aetiology of the low milk-fat syndrome have been proposed, we made use of an acetate source in order to increase milk-fat production in lactating cows.

Six high-producing (25–30 kg milk/d) cows, fitted with rumen cannulas, were used in a change-over design experiment. Three feeding periods of 35 d, each with a collection period of 8 d, were conducted where milk quality and rumen variables were measured. Ammonium acetate was continuously infused directly into the rumen in three concentrations (0, 25 and 50 g/kg diet). Complete diets were given *ad lib*. No detrimental effects on voluntary intake due to ammonium acetate infusion were observed. The results presented in the Table are means of six cows per treatment over the three experiemental periods.

Ammonium acetate (g/kg diet)		Rumen pH	Rumen volatile fatty acids (mol %) C2	C3	C4	Rumen C2:C3	Milk fat (g/kg)	Milk fat (g/d)
0	Mean	5·6[a]	54·7	27·8	12·2	1·97	29[a]	550·8
	SE	0·10	2·27	2·25	1·15	0·23	2·0	45·5
25	Mean	5·6[a]	57·9	25·5	12·5	2·27	34[b]	680·4
	SE	0·05	1·48	1·76	0·08	0·21	1·5	75·1
50	Mean	6·3[b]	58·8	24·9	10·3	2·36	32[a]	519·6
	SE	0·11	3·68	3·03	0·66	0·50	1·7	77·5

[a,b]Mean values in a vertical column with different superscript letters were significantly different ($P<0·05$).

Ammonium acetate progressively increased the C2:C3 ratio and C2 content in the rumen and at 25 g/kg diet increased the fat content significantly. These results suggest that acetate added to diets which produce low milk-fat syndrome may partially prevent this problem. The use of ammonium acetate provides an additional nitrogen source.

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Uptake of ammonia across the liver of forage-fed cattle. By J. C. WILTON and M. GILL, *Institute of Grassland and Animal Production, Hurley, Maidenhead, Berkshire SL6 5LR* and M. A. LOMAX, *Department of Physiology and Biochemistry, University of Reading, PO Box 228, Reading RG6 2AJ*

High rumen ammonia production rates have been implicated in the poor utilization of nitrogen by animals fed on forage diets (Beever & Gill, 1987). Results reported here are based on two studies which consider firstly, ammonia uptake across the liver of animals infused with two levels of ammonia into the mesenteric vein and, secondly, portal and hepatic absorption of ammonia in animals offered silage twice daily.

Three Friesian steers, live weight (LW) approximately 160 kg, were given 21 g dry matter (DM)/kg LW of a pelleted diet (g/kg; 700 dried grass, 300 flaked maize) hourly from a belt feeder. Portal and hepatic flow rates and the metabolism of ammonia and urea by the liver were determined using the procedures described by Lomax *et al.* (1983). Animals were infused at 4-d intervals in a 3×3 Latin-square design with treatments A (1·25 M-sodium chloride), B (0·625 M-NaCl and 0·625 M-NH_4Cl) and C (1·25 M-NH_4Cl) at a rate of 2 ml/min for 3 h. In the second experiment the same animals were given silage at a rate of 22 g DM/kg LW. After 14 d, blood samples were taken at 15 and 30 min intervals for 4 h after feeding. The Table shows the mean ammonia values over the infusion period.

NH_4Cl infusion (M) . . .	0·0	0·625	1·250	SEM	Statistical significance: $P \leq$
Arterial concentration (mM)	0·099	0·118	0·108	0·004	0·009
Portal absorption (mmol/min)	0·951	1·244	2·143	0·073	0·001
Hepatic production (mmol/min)	–0·909	–1·246	–2·183	0·083	0·001
Splanchnic absorption (mmol/min)	0·041	–0·002	–0·040	0·042	0·397

The arterial concentrations of ammonia were slightly increased by the ammonia infusions, however, splanchnic absorption was negative, indicating complete removal by the liver of ammonia arriving in the portal vein. Hepatic urea production was increased from 1·68 mmol/min on treatment A to 3·56 mmol/min on treatment C.

When these animals were given silage, the mean arterial ammonia concentration (0·21 (SEM 0·04) mM) was higher than that during the ammonia infusion experiments. Portal and hepatic production rates could not be measured in all silage-fed animals, but in two animals the portal absorption rates of ammonia (2·35, 1·88 mmol/min) were similar to that during the highest rate of ammonia infusion (treatment C). Splanchnic output was estimated to be higher (0·156 (SE 0·079) mmol/min) indicating that the ability of the liver to remove ammonia from the portal vein may be reduced in cattle fed on silage diets. Mean hepatic production of urea was 2·67 mmol/min on the silage diet.

JCW acknowledges the support of a SERC CASE studentship. This work was also supported by a grant from the AFRC.

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Lipids as rumen-defaunating agents. By C. J. NEWBOLD* and D. G. CHAMBERLAIN, *Hannah Research Institute, Ayr KA6 5HL*

Changes in rumen metabolism associated with the removal of protozoa can lead to an improved supply of nutrients to the host and this can be translated into increased animal production (Bird & Leng, 1984). However, practical application is hindered by the lack of a method of defaunation suitable for routine use on-farm. The experiments reported here examined the potential of dietary inclusion of lipid as a defaunating procedure.

Toxicity of lipid to rumen protozoa was examined in two experiments in vitro. Strained rumen fluid was incubated with the appropriate lipid source for 3 h before measuring protozoal activity by the method of Campbell *et al.* (1982). In the first experiment, free fatty acids at 100 mg/g had the following effects on protozoal activity (expressed as a proportion of the control activity) (mean (SE)): $C_{12:0}$, 0·71 (0·02); $C_{18:0}$, 0·55 (0·07); $C_{18:2}$, 0·14 (0·04) (*n* 4). The second experiment compared coconut oil (rich in C12 and C14 saturated acids) with linseed oil (rich in C18 unsaturated acids) added at 20 μl/ml. The protozoal activities (mean (SE)) were linseed oil, 0·39 (0·01); coconut oil, 0·74 (0·05), confirming the greater toxicity of the C18 unsaturated acids observed in the first experiment.

In an in vivo experiment, four rumen-cannulated sheep given a diet of molassed beet pulp (800 g/d) and hay (200 g/d) in two equal meals were given additions, via the rumen cannula, of coconut oil or linseed oil during 4-week periods. Each oil was added at four levels: 0, 50, 100 and 150 ml/d, the level being increased progressively by 50 ml/d at the end of each week of a 4-week period. Animals were rested for 2 weeks on the control diet between the two experimental periods to allow the protozoal populations to recover. Samples of rumen contents were obtained at the end of each week and the protozoal counts ($\times 10^{-5}$/ml) for the 0, 50, 100 and 150 ml/d additions respectively were (mean (SE)): coconut oil, 5·1 (0·26), 2·5 (0·69), 0, 0; linseed oil, 5·7 (0·40), 4·2 (0·40), 1·9 (0·3), 0·7 (0·06).

In a second in vitro experiment the sheep were given a diet of barley (600 g/d) and hay (400 g/d) and the procedure repeated. Protozoal counts ($\times 10^{-5}$/ml) for the 0, 50, 100 and 150 ml/d additions respectively were: coconut oil, 2·1 (0·22), 0, 0, 0; linseed oil, 2·56 (0·15), 1·38 (0·25), 0·22 (0·15), 0. On this diet both oils reduced intake, however, the effect was more marked and occurred at a lower level of addition with the coconut oil.

In contrast with the results of the in vitro experiments, coconut oil was apparently more toxic than linseed oil in vivo. Whereas linseed oil did not affect intake, coconut oil in amounts >50 ml/d reduced intake markedly. The results indicate a potential application for specific oils as a means of producing severe reductions, if not complete removal, of the protozoal population.

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Observations on how sodium chloride loading of the rumen depresses food intake in sheep.

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Sodium chloride loading of the rumen inhibits food intake in sheep (Ternouth & Beattie, 1971; Phillip *et al.* 1981), but the organ mediating the response has not been identified.

Sheep fed on lucerne (*Medicago sativa*) pellets *ad lib.* were deprived of food for 5·5 h before NaCl loads were injected into the rumen (R; 300 ml) in Expt 1 (5×5 Latin square), and into the rumen (R; 175 ml) or abomasum (A; 50 ml) in Expt 2 (6×6 Latin square). After loading (0 min), intake was recorded at 10, 20, 30, 60 and 90 min. Drinking water was not available from 0 to 60 min.

In Expt 1 there was a linear decrease in intake with increasing NaCl load from 0 to 10 min, but it increased linearly from 60 to 90 min when water was available. Intake was unaffected by NaCl loading of the abomasum (Table 1).

Table 1. *Food intake (g)*

Period of experiment		Expt 1					
(min)	NaCl load, g in rumen . . .	2·37C	6·25	12·5	25	5O	SEM
0–10		321	313	250	253	148	24·4
60–90		56	103	116	144	142	18·9
		Expt 2					
	NaCl load, g in rumen (R) or abomasum (A) . . .	0·41(A)C	7·1(A)	14·3(A)	2·37(R)C	25(R)	5O(R)	SEM
0–10		296[a]	263[a]	250[a]	283[a]	250[a]	93[b]	29·5
60–90		111[a]	136[a]	151[a]	110[a]	152[a]	326[b]	34·2

C, NaCl load to maintain pre-feeding tonicity.

[a, b]Mean values in horizontal rows with different superscript letters were significantly different (Newman-Keuls test): $P<0·05$.

The mean increases in tonicity of rumen contents after 10 min were 249 and 177 mosmol/l with 50 g NaCl loads in Expts 1 and 2 respectively. From 60 to 90 min the tonicities decreased by 146 and 94 mosmol/l due to drinking. Abomasal tonicity increased by 315 mosmol/l, 10 min after a 14·3 g NaCl load. Despite similar mean changes in tonicity of jugular plasma from 0 to 10 min and 0 to 20 min (Table 2), intake was inhibited with 5O(R) but not with either 7·1(A) or 14·3(A). From 60 to 90 min, intake was significantly greater with 5OR than for controls (Expts 1 and 2), despite equal or greater plasma tonicity changes. The observed intake depressions from NaCl loading of the rumen were therefore mediated by the reticulorumen (or omasum) but not by direct effects on the abomasum, liver or brain. The intake response was not ion-specific as it was replicated with equi-osmotic loads of polyethylene glycol-200 into the rumen.

Table 2. *Plasma tonicity changes*

Expt . . .	2		2		2		1	
Period of experiment (min) . . .	0–10		0–20		60–90		60–90	
Treatment (see Table 1)	7·1(A)	5O(R)	14·3(R)	5O(R)	2·37(R)C	5O(R)	2·37(R)C	5O(R)
Tonicity (Δ mosmol/l plasma)	2·7	2·8	7·7	7·8	0·4	0·4	–2·9	3·3

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Effect of feeding frequency on total (sham-feeding) and unilateral parotid (normal-feeding) saliva production in sheep. By R. R. CARTER and W. L. GROVUM, *Department of Biomedical Sciences, University of Guelph, Ontario, Canada N1G 2W1*

Parotid salivary flow is stimulated at the onset of eating, but declines rapidly during the course of the meal (Carr & Titchen, 1978). Offering food frequently may provide a repeated stimulus to parotid secretion. The experiments reported here investigated the effect of feeding frequency on unilateral parotid and total saliva production during eating.

Oesophageal-cannulated sheep (n 5) were sham-fed on lucerne (*Medicago sativa*) hay (890 g/kg dry matter (DM), 236 g crude protein (nitrogen × 6·25)/kg, 412 g acid-detergent fibre/kg) as one (800 g), two (400 g), four (200 g) or eight (100 g) meals/d according to a randomized complete block design replicated four times. Total ingesta were collected from the oesophageal fistulas to calculate total saliva production. Three of these sheep with reversible re-entrant unilateral parotid cannulas (Carter & Grovum, 1988) were similarly offered the same hay with normal feeding (three meals/d). Unilateral parotid saliva was collected during meals.

Frequency of meal (/d) . . .		1	2	4	8	SEM	Statistical significance: $P\leq$
DM intake (g)	Sham	562·4	622·3	629·0	638·5	19·9	NS
	Normal	514·7[a]	579·3[b]	614·2[b]	627·0[b]	18·0	0·017
Duration of meal (min)	Sham	56·9[a]	57·4[a]	70·8[a]	86·0[b]	4·7	0·006
	Normal	64·2[a]	71·3[a,b]	78·0[b]	82·1[b]	3·5	0·035
Saliva production:							
ml	Sham	1553·4[a]	1736·8[b]	1851·3[c]	2086·6[d]	22·3	0·001
	Normal	208·8[a]	247·7[a]	306·9[b]	351·9[b]	15·8	0·003
ml/g DM intake	Sham	2·77[a]	2·80[2]	2·95[a]	3·28[b]	0·09	0·008
	Normal	0·43[a]	0·44[a]	0·51[a]	0·57[b]	0·02	0·006
ml/min	Sham	28·1	31·0	27·0	25·0	1·9	NS
	Normal	3·32[a]	3·48[a]	3·92[a,b]	4·33[b]	0·15	0·011
ml/g DM intake per min (× 10^2)	Sham	5·0[a]	5·0[a]	4·3[a,b]	3·9[b]	0·22	0·014
	Normal	0·69	0·61	0·64	0·69	0·03	NS

NS, not significant.

[a–d]Mean values in horizontal rows with different superscript letters were significantly different: $P<0·05$.

Increasing feeding frequency from one to eight meals led to a 34% and a 68% increase in total and unilateral parotid saliva production respectively. The response in both cases was linear but the sham-feeding results also had curvilinearity at the lower feeding frequencies ($P<0·05$). The remaining significant responses were also linear except that the DM intake results for normal feeding had curvilinearity at the high frequencies. Simple ratio adjustment of saliva production for DM intake and time spent eating suggested that the saliva response to feeding frequency was mediated by increases in DM intake and eating time. However, a more comprehensive regression model indicated that there were effects of feeding frequency in addition to DM intake and eating time responsible for the increased parotid and total saliva production.

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Lipolysis and fat cell size in subcutaneous adipose tissue from women breast-feeding and those artificially feeding their babies. By D. M. CAMPBELL, *Department of Obstetrics and Gynaecology, University Medical Buildings, Aberdeen AB9 2ZD*, and M. A. RADCLIFFE and A. D. SMITH, *Department of Physiology, University of Aberdeen, Marischal College, Aberdeen AB9 1AS*

Loss in body-weight is reported to be greater in women breast-feeding than in those artificially feeding their babies during the period 1–13 weeks postnatally (Dennis & Bytheway, 1965). While measurements of total body water and potassium, of skinfold thickness and of fat cell size indicate that net lipid mobilization from white adipose tissue (WAT) contributes to this loss (Pipe *et al.* 1979), the results have not been considered in the context of lactational status. We have now investigated cell size and rates of lipolysis in WAT from women breast-feeding or artificially feeding their babies.

Body-weight and serum levels of oestradiol-17β (E_2), progesterone (PROG), insulin (INS) and prolactin (PRL) were measured serially at 38 weeks of pregnancy, 3–6 d postnatally and 5–6 weeks postnatally (visits 1–3 respectively) in normal women who fed their infants either artificially (group A) or from the breast (group B), and who did not receive oral contraceptives. Small pieces of WAT were taken from the buttock by percutaneous needle biopsy and incubated at 37° in HEPES-buffered Krebs saline containing bovine albumin (40 g/l) and glucose (5 mM), pH 7·4, to measure basal and isoprenaline (10^{-5}M)-stimulated lipolysis. Fat cell diameters were measured by microscopy (*n* 150–200). Samples were obtained from fed subjects at 09.00–10.00 hours.

	Group A, artificial feeding (*n* 6)						Group B, breast-feeding (*n* 9)					
Visit . . .	1		2		3		1		2		3	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Body-wt (kg)	77·0	8·2	70·6	7·6	67·0	7·4	73·2	9·9	66·6	7·9	62·1	7·5
Serum hormones ($\log_e$):												
hPL (μg/ml)	2·02	0·26	—	—	—	—	2·12	0·18	—	—	—	—
E_2 (nmol/l)	4·06	0·18	–2·11	0·27	–2·28	0·31	4·34	0·30	–2·14	0·32	–2·39	0·07
PROG (nmol/l)	6·70	0·41	2·03	0·49	1·20	0·48	6·73	0·40	1·91	0·35	1·34	0·29
INS (μU/ml)	3·77	0·86	2·63	0·21	2·40	0·19	3·22	0·54	2·71	0·19	2·48	0·27
PRL (μU/ml)	8·10	0·31	7·50	0·54	5·30	0·49	8·50	0·45	8·20	0·61	6·40	1·08
Fat cell diameter (μm)	112·0	5·0	109·0	6·0	99·0	11·0	98·0	10·1	106·0	12·0	101·0	8·1
Lipolysis*:												
Basal	0·67	0·52	0·68	0·34	0·61	0·47	0·60	0·49	0·56	0·20	0·44	0·34
isoprenaline-stimulated increment	2·92	0·39	2·41	0·87	2·61	0·83	2·74	1·17	2·99	0·75	2·65	0·87

hPL, human placental lactogen.
*nmol glycerol liberated/mg fat in 90 min.

Body-weight and serum hormone levels followed established patterns. A significant inter-group difference arose for PRL only at visit 3 ($P<0·05$). Fat cell mean diameter did not differ between the groups postnatally; but at late pregnancy it was lower in group B than in group A ($P<0·01$), and it increased significantly in the visit 1–2 interval ($P<0·02$). There were no inter-group or inter-visit differences in the rates of basal or catecholamine-stimulated lipolysis.

We conclude that the characteristics of body fat (as indicated by fat cell size and rates of basal and catecholamine-stimulated lipolysis in subcutaneous WAT) are similar in lactating and non-lactating women during the first 5–6 weeks postnatally.

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The effect of long-term administration of bovine somatotrophin on plasma hormone and metabolite levels in dairy heifers during their first lactation. By R. F. BUTTERWICK[1], T. E. C. WEEKES[1], P. ROWLINSON[2], D. S. PARKER[1] and D. G. ARMSTRONG[1], [1]*Department of Agricultural Biochemistry and Nutrition* and [2]*Department of Agriculture, The University, Newcastle upon Tyne NE1 7RU*

Previous studies have shown that long-term administration of bovine somatotrophin (BST) to dairy cows significantly improves lactational performance (Bauman *et al.* 1985). The objectives of this study were to assess the effects of long-term daily administration of BST to dairy heifers on lactational performance and on the plasma levels of key hormones and metabolites.

Friesian dairy cows in their first lactation were allocated at calving to one of three treatments: daily subcutaneous administration of recombinantly derived BST (25 mg/d) from either week 2 (BST 2; *n* 6) or week 10 (BST 10; *n* 6) of lactation, or injection of buffer solution from week 2 of lactation (C; *n* 7). Treatments continued until week 42 of lactation. Blood samples were taken over 24 h-periods on days 11, 19 and 50 post-calving and every 30 d thereafter. Initially cows were housed and individually fed *ad lib.* on a complete diet (concentrate 0·6: grass silage 0·4, on a dry matter basis). In mid-lactation, cows were turned out to grass but continued to receive concentrates depending on milk yield and body condition.

BST treatment had no effect on mean body-weights but resulted in the significant loss of body condition score from the BST 2 group during early lactation (Butterwick *et al.* 1988). The Table shows mean daily milk yields over weeks 3–40 of lactation and plasma hormone and metabolite levels meaned over all sampling days. Treatment with BST significantly ($P<0·01$) increased plasma growth hormone (GH) levels.

Group . . .	C	BST 10	BST 2	SE
Milk yield (kg/d)	17·4	20·5*	20·3*	1·0
GH (ng/ml)	6·4	16·5**	18·9**	2·3
Insulin (μU/ml)	10·4	11·1	10·4	0·8
Glucose (mM)	3·84	3·93	3·80	0·04
NEFA (mM)	0·22	0·28*	0·27*	0·02
3-Hydroxybutyrate (mM)	0·43	0·42	0·47	0·03

NEFA, non-esterified fatty acids.
Significantly higher than the control value: *$P<0·05$, **$P<0·01$.

Although BST administration had no significant effect on mean insulin levels over the treatment period, BST 2 cows did exhibit significantly ($P<0·05$) lower insulin levels during early lactation. Treatment with BST resulted in significantly ($P<0·05$) higher NEFA concentrations but had no effect on 3-hydroxybutyrate, glucose, urea or α-amino nitrogen levels or on the levels of throxine and triiodothyronine. It appears that the changes observed in key metabolites and hormones were a result of altered energy status associated with BST administration.

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