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Endocrine control of milk protein production in well-fed dairy cows

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ABSTRACT

A hyperinsulinemic-euglycemic clamp was used to investigate the effect of insulin on milk protein synthesis in well-fed Holstein cows ($n=4$) with or without additional amino acids. Cows were fed a dry total mixed ration diet formulated to exceed requirements for metabolizable energy and protein. During abomasal water infusion, the insulin clamp increased milk protein yields by 15% (+128 g/d; $P<0.01$); when combined with abomasal infusion of casein plus branched-chain amino acids, milk protein yield was increased by 25% (+213 g/d; $P<0.01$). Concentrations of casein and whey proteins in milk were increased by insulin clamp treatments, with little change in their relative proportions. Providing amino acids without the insulin clamp had no effect on any production variables. Results confirm the important regulatory role of the endocrine system in milk protein synthesis and demonstrate the potential to produce milk protein is not fully expressed.

Keywords: Milk protein; insulin; amino acids; dairy cows.

INTRODUCTION

Protein is the most valuable gross component in milk (Valeur, 1997) and its value is increasing from both economic and nutritional perspectives. This has led to a research emphasis on increasing the concentration and yield of milk protein from dairy cows. Most research has focused on the effects of amino acid (AA) supply on milk protein production (e.g., Rulquin *et al.*, 1995). In contrast, relatively little is known about the physiological aspects of the regulation of milk protein production; most research on the endocrine regulation of lactation has primarily focused on lactogenesis (Tucker, 1985). Recently, however, our group demonstrated that milk protein concentration and yield by well-fed cows can be markedly enhanced by manipulation of endocrine signals (McGuire *et al.*, 1995; Griinari *et al.*, 1997).

The current study used a hyperinsulinemic-euglycemic clamp in combination with postprandial supply of casein and branched-chain AA (BCAA) to examine the separate and combined effects of insulin and AA on milk protein yield. The objective was to examine treatment effects on the milk protein yield of well-fed cows. This contrasts with many studies in which attempts were made to restore milk protein levels in cows fed a diet supplying inadequate or marginal amounts of certain AA, metabolizable energy, or both (e.g., Ørskov *et al.*, 1977).

MATERIALS AND METHODS

Details of this study, including ingredients and nutrient composition of the diet, and technical details of the abomasal infusions, the abomasal infusion procedure and the insulin clamp technique have been described previously (Mackle *et al.*, 1999b; Mackle *et al.*, 2000). Briefly, the study involved four lactating, pregnant, multiparous, rumen-fistulated Holstein cows that were 220 ± 11 days in milk and averaged 641 ± 40 kg live weight (mean \pm SD). Cows were fed a dry total mixed ration (ad libitum), based on alfalfa hay and concentrates, and formulated to exceed requirements for metabolizable energy, metabolizable protein, and essential AA (EAA) using the Cornell Net Carbohydrate and Protein System (Ithaca, NY, USA).

Automatic feeders delivered equal portions of feed at 2-h intervals to minimise postprandial fluctuations in nutrient supply.

Treatments were: 1) abomasal infusions of water (6.0 L/d); 2) abomasal infusions of casein (500 g/d) plus BCAA (88 g/d) (CB) 3) water infusion plus insulin clamp (Water+I) and 4) CB infusion plus insulin clamp (CB+I).

Cows were milked at 0600 and 1800 h. At each milking, milk yield was recorded and milk samples collected from each cow. Milk was analysed for protein and fat using an infrared milk analyser (Milkoscan 4000; Foss Electric, Hillerød, Denmark). Composite samples were analysed for milk nitrogen fractions by macro-kjeldahl techniques (AOAC, 1995). Blood samples were obtained from jugular catheters at 6-h intervals during d-3 and d-4 of the baseline interval and at hourly intervals for all 4 d of the insulin clamp. Plasma was harvested and stored at -20°C and concentrations of insulin, IGF-I, IGF-BP and glucose were determined as described by Mackle *et al.* (1999a, b).

Mean values for each cow were determined across the 4 d of each baseline interval (Water and CB treatments), and on the last day of the insulin clamp (Water+I and CB+I treatments). Data were analysed using SAS PROC MIXED with cow and period as random effects, and insulin treatment and CB treatment as fixed effects in the model. Data are presented as least squares means. The PDIFFS option in SAS 6.12 was used to generate comparisons between individual treatment least squares means.

RESULTS

The insulin clamp increased circulating insulin levels to almost fourfold baseline levels, while blood euglycemia was maintained by infusion of exogenous glucose (Figure 1). Mean DMI was 26.0 ± 1.2 kg/d and was marginally reduced (1.1 kg/d; $P = 0.09$) during the insulin clamp treatments. Milk yield was enhanced during the insulin clamps; cows receiving the CB+I treatment produced 12.5% (3.3 kg/d; $P<0.02$) more milk than those on Water treatment. The greatest concentration and yield of milk protein occurred when cows received the CB+I treatment and milk protein yield had not reached a plateau by d-4 of

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the insulin clamp. Cows on the CB+I treatment produced milk with 11% higher ($P<0.01$) protein concentration and when combined with the increased milk yield, this resulted in 25% more milk protein compared to the control (Table 1).

Milk fat concentration was reduced by 14% following the insulin clamp (-0.45% units; $P<0.01$; Table 1). However, the yield of milk fat was reduced (-0.08 kg/d; $P<0.01$) only when the insulin clamp was combined with abomasal infusion of Water (interaction, $P<0.09$). The concentration of casein and whey protein in milk were elevated ($P<0.01$) by 7.0 and 10.0% respectively, for the insulin clamp treatments compared to the Water treatment. There were no effects ($P>0.1$) of infusion of casein plus BCAA on any of the variables measured.

Concentrations of insulin and IGF-I in milk and plasma were increased ($P<0.01$) during the insulin clamp treatments. Increases in plasma and milk were proportional so that the plasma to milk ratios for these hormones were unaltered ($P>0.1$; Table 2). Plasma concentrations of IGFBP-2 were reduced ($P<0.05$) by insulin clamp treatments with levels decreasing by an average of 68% compared to the water treatment (Table 2); none of the other IGFBP were affected by treatments.

TABLE 1: Least squares means for DMI, milk yield and milk composition during either abomasal infusions of water or casein plus BCAA (CB), water infusion plus insulin clamp (Water+I) and CB infusion plus insulin clamp (CB+I). Data from Mackle *et al.* (1999b).

Variable	Treatment				P ¹	
	Water	CB	Water+I	CB+I	SEM	INS
DMI, ² kg/d	26.2	27.6	25.1	25.2	1.2	0.09
Milk yield, kg/d	26.5 ^b	27.5 ^b	28.3 ^{ab}	29.8 ^a	2.4	0.02
Milk protein						
%	3.29 ^b	3.31 ^b	3.52 ^a	3.66 ^a	0.185	0.001
kg/d	0.867 ^c	0.895 ^c	0.995 ^b	1.080 ^a	0.073	0.001
Casein, %	2.49 ^c	2.54 ^{bc}	2.63 ^{ab}	2.73 ^a	0.13	0.01
Whey protein, %	0.66 ^c	0.65 ^c	0.70 ^b	0.74 ^a	0.05	0.001
Milk fat						
%	3.25 ^a	3.15 ^a	2.71 ^b	2.79 ^b	0.110	0.01
kg/d	0.855 ^a	0.856 ^a	0.768 ^b	0.829 ^a	0.065	0.01

^{a,b} Least squares means within rows with different superscripts differ ($P<0.05$).

¹ Main effect of insulin treatment. There were no main effects of abomasal infusions or interactions with insulin clamp treatment.

² Excludes abomasal infusions and intravascular glucose infusions.

TABLE 2. Least squares means for plasma metabolite and hormone concentrations and milk hormone concentrations during either abomasal infusions of water or casein plus BCAA (CB), water infusion plus insulin clamp (Water+I) and CB infusion plus insulin clamp (CB+I).

Variable	Treatment				P ¹	
	Water	CB	Water+I	CB+I	SEM	INS
Insulin						
Plasma, ng/ml	2.7 ^b	2.8 ^b	11.7 ^a	10.3 ^a	1.30	0.001
Milk, ng/ml	1.5 ^b	1.6 ^b	4.3 ^a	4.4 ^a	0.29	0.001
Plasma:Milk	1.9	2.1	2.8	2.3	0.41	NS ²
IGF-I						
Plasma, ng/ml	101.6 ^b	103.4 ^b	132.2 ^a	131.1 ^a	11.9	0.001
Milk, ng/ml	3.8 ^b	3.6 ^b	5.0 ^b	4.4 ^{ab}	0.49	0.01
Plasma:Milk	27.3	29.6	26.6	30.1	1.89	NS
IGFBP-2, ³	2846 ^a	1253 ^{ab}	944 ^b	997 ^b	546	0.05

^{a,b,c} Least squares means within rows with different superscripts differ ($P<0.05$).

¹ Main effect of insulin treatment. There were no main effects of abomasal infusions or interactions with insulin clamp treatment.

² NS = non significant at $P>0.1$.

³ Values for IGF binding proteins (IGFBP) are arbitrary densitometer units from phosphoimager scanning of Western ligand blots.

FIGURE 1: Temporal pattern of plasma insulin, blood glucose, and glucose infusion rate for a 48-h baseline period followed by a 96-h hyperinsulinemic-euglycemic clamp. Insulin was infused at a constant rate of 1mg/kg BW⁻¹/h⁻¹ and glucose was infused at variable rates as required to maintain blood euglycemia. The dashed lines on the blood glucose panel represent $\pm 10\%$ of baseline glucose levels. Values are means of four cows and the standard error of the means were 1.1 ng/ml and 0.8 mg/dl for plasma insulin and blood glucose concentrations, respectively. Data from Mackle *et al.* (1999b).

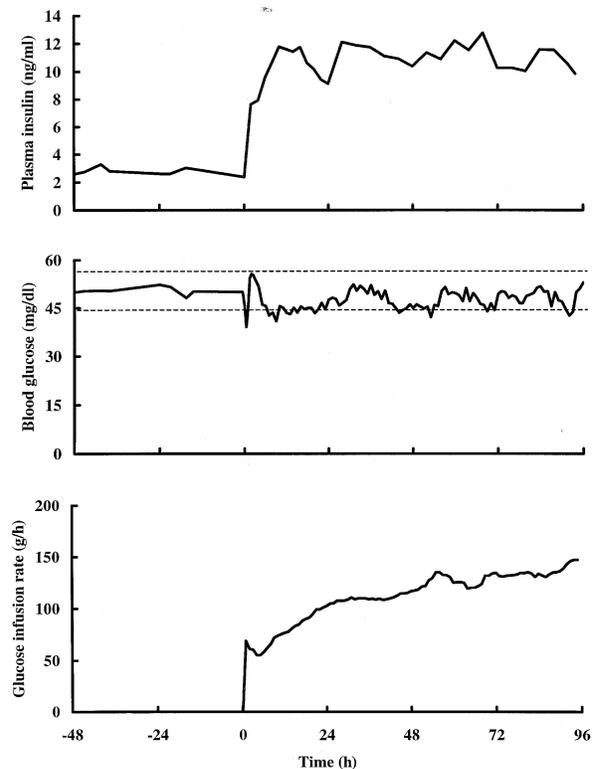
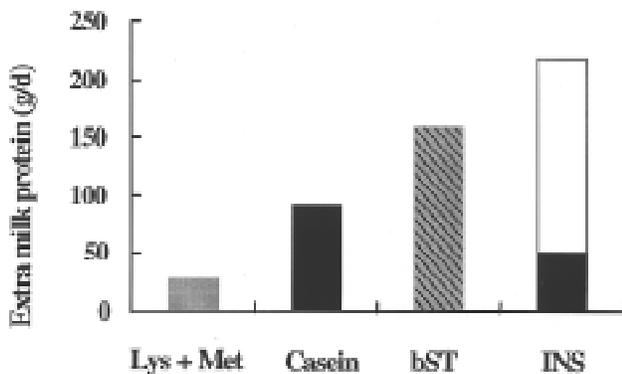


FIGURE 2: Milk protein response to various treatments. Lys + Met response is the average response to supplementation of Lys, Met, or both, from Rulquin *et al.*, (1995) for 121 studies; casein represents the average response of 35 experiments presented in 26 published reports (Bauman and Mackle, 1997); bST response is an average theoretical response based on an increase of 5 kg milk/day at 3.2% protein (Bauman and Vernon, 1993). Insulin clamp plus abomasal infusion of casein response (INS; sum of shaded and open areas) represents average of two studies (Griinari *et al.*, 1997; Mackle *et al.* 1999b, 2000) with shaded area being the response to casein infusion alone.



DISCUSSION

Several groups have made attempts to evaluate the effects of insulin on lactation, but the effects of hypoglycemia (e.g., Schmidt, 1966; Léonard and Block, 1997) have confounded their efforts. Other studies failed to raise circulating insulin concentrations (Metcalf *et al.*, 1991) or have maintained the elevated insulin levels for only a few hours (Laarveld *et al.*, 1981). In the current study, elevation (~4x) of circulating insulin concentrations over a 96-h period (Figure 1) allowed us to evaluate the effect of insulin on the chronic control of milk protein production in well-fed cows.

The 25% increase (+213 g/d) in milk protein observed during the CB+I treatment was similar to previous results (+230 g/d; Griinari *et al.*, 1997). The 11% increase in milk protein concentration during the CB+I treatment is impressive (Table 1), especially since cows were already in a well-fed state. Increases in milk protein concentration of this magnitude are normally observed only where treatments restore milk protein to normal levels by overcoming dietary energy or protein (AA) deficiencies (e.g., Ørskov *et al.*, 1977). The increase in milk protein of 128 g/d (+15%) associated with the Water+I treatment was greater than the +4-7% response observed previously (McGuire *et al.*, 1995; Griinari *et al.*, 1997). It is likely that the difference relates to a more adequate supply of absorbed AA in the current study; we observed only a small reduction in DMI (Table 1) during the insulin clamp, which contrasts with earlier studies (McGuire *et al.*, 1995; Griinari *et al.*, 1997). Consistent with this, abomasal infusion of casein plus BCAA gave only a small non-significant milk protein response in the present study (+28 g/d) whereas infusion of casein gave a significant increase of 60-80 g/d in our previous studies (Griinari *et al.*, 1997; Mackle *et al.*, 1999a).

We also observed a galactopoietic effect of the insulin clamp (Table 1). On average, cows received nearly 20% of their total net energy inputs from infused glucose during

the insulin clamp. However, infused glucose is not likely to have stimulated the additional yield of milk or protein, as many studies have shown that provision of glucose to well-fed cows does not increase these variables (e.g. Léonard and Block, 1997). Furthermore, insulin does not cause an acute increase in the uptake of glucose by the mammary gland in ruminants (e.g. Laarveld *et al.*, 1981). It seems likely that the increase in milk and protein yield during the insulin clamp resulted from a stimulatory effect on mammary secretory cells, however, the mechanism(s) responsible is not clear.

Milk insulin concentrations gradually increased over the 4-day insulin clamp and this increase paralleled the temporal pattern for the increase in milk protein (data not shown). The movement of insulin from plasma to milk may involve transcellular pathways as suggested for IGF-I (Prosser *et al.*, 1991), and this could be consistent with a direct effect of insulin on mammary secretory cell activity and milk protein synthesis. Insulin is an important hormone in the development of the mammary gland, and mammary epithelial cells possess insulin receptors whose numbers appear to correspond to mammary activity (Collier *et al.*, 1989). A direct role for insulin in the regulation of milk protein synthesis during established lactation seems possible.

The effects of insulin could be indirect and involve elements of the IGF system as possible mediators. As in earlier insulin clamps (McGuire *et al.*, 1995; Griinari *et al.*, 1997), we observed that the circulating concentration of IGF-I was increased and IGF-BP-2 was decreased during the insulin clamp (Table 2). We also found that during the insulin clamp, milk concentrations of IGF-I were increased and the increase in IGF-I of plasma and milk paralleled the increase in milk protein yield (data not shown). Mammary epithelial cells have receptors for IGF-I and the receptor numbers increase with the onset of lactation (Collier *et al.*, 1989; Cohick, 1998). Furthermore, intravenous infusions of IGF-I result in a galactopoietic response (e.g., Prosser *et al.*, 1994) and IGF-I is thought to be involved in the lactational response to bST (Bauman and Vernon, 1993). However, these responses differ from those observed during the insulin clamp. Studies involving close arterial infusion of IGF-I demonstrate effects on milk yield are acute and correspond to increases in blood flow (Cohick, 1998). During close arterial infusion of IGF-I and treatment with bST, the lactational responses involve increases in the yield of milk and milk components so that the composition is generally unaltered. In contrast, the lactational response to the insulin clamp is gradual, and, although there is a modest increase in milk yield, the predominant characteristic is an increase in milk protein content (McGuire *et al.*, 1995; Griinari *et al.*, 1997; Table 1).

Subsequent studies utilising the insulin clamp technique have failed to demonstrate stimulatory effects of insulin on milk protein yield in dairy cows (Annen *et al.*, 1998) and sheep (Back *et al.*, 1998). Reasons for these observations are not clear, however it is possible that the reduction in DMI observed in both studies (-11%, Annen *et al.*, 1998; -17%, Back *et al.*, 1998) during the insulin clamp, contributed. However, substantial reductions in DMI were observed during our previous insulin clamps (-25%;

McGuire *et al.*, 1995; -18%, Griinari *et al.*, 1997) but milk protein responses were still apparent. Back *et al.* (1998) suggested that their results could relate to nutritional limitations of the pasture diet used or a fundamental difference between ovine and bovine species. This raises the question as to whether the pasture diet is capable of providing an adequate supply of nutrients for milk protein responses to be expressed during insulin clamp experiments.

Finally, various approaches have been used to enhance milk protein yields in lactating dairy cows, and these are compared in Figure 2. It should be pointed out that the responses which are plotted involve substantial differences in the number of studies ranging from 121 studies for Lys and Met supplementation (Rulquin *et al.*, 1995) to only two studies for the insulin clamp (Griinari *et al.*, 1997; Table 1). Clearly, data contained in this figure demonstrate the potential that exists to increase milk protein yield in well-fed cows by manipulation of endocrine control signals. However, in order for our results to be applied, the underlying mechanism(s) responsible for these effects needs to be elucidated.

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REFERENCES

- Annen, E.L., McGuire, M.A., Hanson, T.W. and Bauman, D.E. 1998. Milk protein production in cows subjected to abomasal infusion of branched-chain amino acids (BCAA) and a hyperinsulinemic-euglycemic clamp. *Journal of Dairy Science*. **76**: (supplement) 354.
- Association of Official Analytical Chemists, International. 1995. Official Methods of Analysis. Vol. II 16th ed. AOAC, Arlington, VA.
- Back, P.J., Mackenzie, D.D.S., Davis, S.R. and Harris, P.M. 1998. The effects of Insulin-nutrient supply interactions on ewe lactation. *Proceedings of the New Zealand Society of Animal Production*. **58**: 205-208.
- Bauman, D.E. and Vernon, R.G. 1993. Effects of exogenous bovine somatotropin on lactation. *Annual Review of Nutrition*. **13**: 437-461.
- Bauman, D.E. and Mackle, T.R. 1997. Amino acid supply and the regulation of milk protein synthesis. Proceedings of the Cornell Nutrition Conference for Feed Manufacturers, Rochester, NY. Cornell Univ., Ithaca, NY. 59: 196-207.
- Cohick, W.S. 1998. Role of the insulin-like growth factors and their binding proteins in lactation. *Journal of Dairy Science*. **81**: 1769-1777.
- Collier, R.J., Ganguli S., Menke P.T., Buonommo F.C., McGrath M.F., Kotts C.E. and Krivi G.G. 1989. Changes in insulin and somatomedin receptors and uptake of insulin, IGF-I and IGF-II during mammary growth, lactogenesis and lactation. In *Biotechnology in Growth Regulation*. (Ed. Heap, R.B., Prosser, C.B., and Lamming, G.E.) 153-163. London, UK: Butterworth & Co.
- Griinari, J.M., McGuire M.A., Dwyer D.A., Bauman D.E., Barbano D.M., and House W.A. 1997. The role of insulin in the regulation of milk protein synthesis in dairy cows. *Journal of Dairy Science*. **80**: 2361-2371.
- Laarveld, B., Christensen D.A. and Brockman R.P. 1981. The effect of insulin on net metabolism of glucose and amino acids by the bovine mammary gland. *Endocrinology*. **108**: 2217-2221.
- Léonard, M. and Block E. 1997. Effects on nutrient and hormonal profile of long-term infusions of glucose or insulin plus glucose in cows treated with recombinant bovine somatotropin before peak milk yield. *Journal of Dairy Science*. **80**: 127-143.
- Mackle, T.R., Dwyer D.A. and Bauman D.E. 1999a. Effects of branched-chain amino acids and casein on milk protein concentration and yield from dairy cows. *Journal of Dairy Science*. **82**: 161-171.
- Mackle, T.R., Dwyer D.A., Ingvarsten K.L., Chouinard P.Y., Lynch J.M., Barbano D.M. and Bauman D.E. 1999b. Effects of insulin and amino acids on milk protein concentration and yield from dairy cows. *Journal of Dairy Science*. **82**: 1512-1524.
- Mackle, T.R., Dwyer D.A., Ingvarsten K.L., Chouinard P.Y., Ross D.A. and Bauman D.E. 2000. Effects of insulin and postprandial supply of protein on use of amino acids by the mammary gland for milk protein synthesis. *Journal of Dairy Science*. **83**: 93-105.
- McGuire, M.A., Dwyer D.A., Harrell R.J. and Bauman D.E. 1995. Insulin regulates circulating insulin-like growth factors and some of their binding proteins in lactating cows. *American Journal of Physiology*. **269**: E723-E730.
- Metcalf, J.A., Sutton J.D., Cockburn J.E., Napper D.J. and Beever D.E. 1991. The influence of insulin and amino acid supply on amino acid uptake by the lactating bovine mammary gland. *Journal of Dairy Science*. **74**: 3412-3420.
- Ørskov, E.R., Grubb D.A. and Kay R.N.B. 1977. Effect of postprandial glucose or protein supplementation on milk yield and composition in Friesian cows in early lactation and negative energy balance. *British Journal of Nutrition*. **38**: 397-405.
- Prosser, C.G., Davis S.R., Farr V.C., Moore L.G., and Gluckman P.D. 1994. Effects of close-arterial (external pudic) infusion of insulin-like growth factor-II on milk yield and mammary blood flow in lactating goats. *Journal of Endocrinology*. **142**: 93-99.
- Prosser, C.G., Fleet I.R., Davis A.J. and Heap R.B. 1991. Mechanism of secretion of plasma insulin-like growth factor-I into milk of lactating goats. *Journal of Endocrinology*. **131**: 459-466.
- Rulquin, H., Verite R., Guinard G. and Pisulewski P.M. 1995. Dairy cows' requirements for amino acids. In *Animal Science Research and Development. Moving toward a new century*. (Ed. M. Ivan) 143. Canadian Society of Animal Science. Ottawa, ON, Canada.
- Schmidt, G.H. 1966. Effect of insulin on yield and composition of milk of dairy cows. *Journal of Dairy Science*. **49**: 381-385.
- Tucker, H.A. 1985. Endocrine and neural control of the mammary gland. In *Lactation*. (Ed. B. L. Larson). 39-79. Ames, Iowa: The Iowa State University Press.
- Valeur, J. 1997. Milk protein production and market prospects. In *Milk composition, Production and Biotechnology* (Ed. Welch, R.A.S., Burns, D.J.W., Davis, S.R., Popay, A.I., and Prosser, C.G.) 93-103. Cambridge, U.K.: CAB International.