DIETARY REGULATION OF PLASMA INSULIN AND GROWTH HORMONE CONCENTRATIONS IN SHEEP

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Abstract

Plasma insulin and growth hormone concentrations have been measured by radio-immunoassay in fasting sheep and in sheep fed either a restricted amount of a concentrate diet or virtually *ad libitum* a variety of dried forage diets providing a wide range of energy and protein intakes.

The plasma insulin concentration of sheep on each diet was positively correlated with the amount of organic matter digested in the alimentary tract ($r = 0{\cdot}74$) and to the amount of crude protein digested in the intestines ($r = 0{\cdot}74$), but was less clearly correlated with the ruminal production of the individual volatile fatty acids (acetate, $r = 0{\cdot}45$; propionate, $r = 0{\cdot}51$; butyrate, $r = 0{\cdot}40$). Plasma insulin was poorly correlated with the plasma glucose concentration ($r = 0{\cdot}28$), but in a group of sheep fed on ryegrass diets it was closely related to the glucose entry rate.

The plasma insulin concentration was also correlated with plasma concentrations of valine, tyrosine, isoleucine, and phenylalanine.

The results suggest that the amount of protein digested in the intestine is the main factor in dietary regulation of the plasma insulin concentration of sheep fed virtually *ad libitum*.

Plasma growth hormone concentrations were negatively correlated with the amount of organic matter digested in the alimentary tract ($r = -0{\cdot}62$), with the amount of crude protein digested in the intestines ($r = -0{\cdot}63$), and with the plasma insulin concentration ($r = -0{\cdot}71$). It is suggested that the negative correlation with plasma insulin concentration reflects the existence of a negative feedback system between the rate of glucose utilization and growth hormone secretion.

I. Introduction

In ruminants, little glucose is absorbed from the gastrointestinal tract, and the plasma glucose concentration changes little after feeding. Nevertheless, glucose plays an important role in ruminant metabolism, and the rate of glucose turnover is dependent on the dietary intake of energy and crude protein (Ford 1965; Judson and Leng 1968; Steel and Leng 1968). Endocrine regulation of metabolism is undoubtedly as important in ruminants as in other species, but the extent to which the digestive tract influences hormone secretion is not known. Consequently, the

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finding that, in sheep, secretion of insulin can be stimulated by intravenous infusion of some volatile fatty acids (VFA) (the main end products of carbohydrate digestion in ruminants) as well as by intravenous infusion of glucose (Manns and Boda 1967; Horino *et al.* 1968) has led to speculation that VFA production and absorption plays an important role in the regulation of plasma insulin concentration (Manns, Boda, and Willes 1967). However, there is no direct evidence that VFA produced in the rumen are more important determinants of insulin secretion than other end products of digestion.

The observations reported here provide evidence on the roles of VFA and other end products of digestion in the regulation of insulin and growth hormone concentrations in the blood. In addition, the relationship between the plasma insulin concentration and the rate of glucose production has been examined.

II. Experimental

(*a*) *Sheep*

Twenty-two Merino wethers, fitted with cannulae in the rumen and abomasum, were maintained indoors in metabolism cages. They were treated to eliminate helminths and received vitamins A and D_3 at regular intervals.

(*b*) *Experimental Design*

A total of 27 diets was fed to groups of three or more animals. Except in the case of two concentrate diets, each diet was offered *ad libitum* for a period of 3 weeks. Then for 2–3 weeks the sheep were fed approximately 90% of the *ad libitum* intake, in equal portions every 3 hr using a mechanical feeder. During the second period measurements of various parameters of digestion were made, and towards the end of the period blood was taken from each sheep by jugular venipuncture on three occasions at intervals of 2 hr. To provide observations on fasted sheep, blood samples were taken from three animals after a fast of 3 days. Blood was centrifuged at 4°C to separate the plasma. Plasma was stored at −20°C. Heparin was used as an anticoagulant.

(*c*) *Diets*

Dried forages varying greatly in food value were prepared from four different grass and three legume species by harvesting each of them at a number of different stages of maturity.

In addition two groups of sheep were fed 500 g daily of a high protein diet (50% lucerne hay, 40% maize, and 10% peanut meal) or a low protein diet (50% wheaten hay and 50% maize).

Details of the diets, and of digestion studies on them are reported elsewhere (Hogan and Weston 1967*a*, 1967*b*, 1969; Hogan, Weston, and Lindsay 1969; Weston and Hogan 1967, 1968*a*, 1968*b*, 1971).

The dried forage and concentrate diets provided a wide range of nutrient intake and feed consumption.

Nitrogen intake was in the range 4–50 g/day, organic matter intake in the range 360–1240 g/day, and digestible organic matter intake in the range 250–970 g/day. Expressed as a percentage of total organic matter the composition of the diets varied from 39 to 60% cell wall constituents, from 6 to 32% crude protein, and from 5 to 18% soluble carbohydrate.

(*d*) *Glucose Entry Rates*

To provide measurements of the glucose entry rate during the feeding of ryegrass diets, the sheep fed early, mid-season, and late season diets at near *ad libitum* levels (Weston and Hogan 1968*b*) were infused intravenously with uniformly labelled [^{14}C]glucose (Radiochemical Centre, Amersham, England). The labelled glucose was given through a catheter at a rate of approximately 0·14 μCi/min for 4 hr after a priming injection of 15 μCi. The catheter used was placed in the left jugular vein at least 1 day before the experiment. During these experiments the

animals were fed at 1·5-hr intervals. Blood samples were obtained from the right jugular vein by venipuncture at intervals of approximately 20 min, commencing 40–60 min after the start of infusion.

The experiments were repeated on the three sheep fed the early season ryegrass after the amount fed had been reduced so that they had been receiving approximately 50% of the *ad libitum* intake for 5 days and again after they had been fasted for 3 days.

(*e*) *Chemical Analyses*

The concentration of insulin in plasma was measured by radio-immunoassay using either a double-antibody method (Hales and Randle 1963) as modified by Bassett and Wallace (1966) or a method using an adsorbent, talc, to separate free [^{125}I]insulin from antibody-bound labelled hormone (Rosselin *et al.* 1966); the procedure is described by Bassett and Thorburn (1971).

Plasma growth hormone concentrations were determined by a similar radio-immunoassay method (Wallace and Bassett 1970).

Plasma glucose concentrations were determined by the glucose oxidase method of Huggett and Nixon (1957). The specific radioactivity of plasma glucose was determined by isolating glucose from deproteinized plasma as the glucosazone, after adding carrier glucose. The osazones were recrystallized twice, oxidized by the oxygen flask method (Kalberer and Rutschmann 1961) as modified by Downes *et al.* (1970), and the liberated CO_2 absorbed in 12 ml of an ethanolamine : 2-methoxyethanol (1 : 9) mixture. 10 ml of the ethanolamine mixture containing absorbed CO_2 and 9 ml scintillation solution [0·3% diphenylbenzene and 0·01% 1,4-bis(5-phenyloxazol-2-yl)benzene (w/v) in toluene] were added to vials and the radioactivity of the CO_2 determined in a liquid scintillation spectrometer (Packard Instruments Ltd., Illinois).

Plasma concentrations of free amino acids were determined in the way described by Hogan, Weston, and Lindsay (1968).

(*f*) *Calculations*

The calculation of digestible organic matter intake (DOM intake), nitrogen in forms other than ammonia digested in the intestines (NAN), rate of flow of digesta from the abomasum, ruminal VFA production, and values for these parameters are reported elsewhere (Hogan and Weston 1967*a*, 1967*b*, 1969; Weston and Hogan 1967, 1968*a*, 1968*b*; Hogan, Weston, and Lindsay 1969).

The mean plasma hormone and glucose concentrations for the three to six animals fed each diet were calculated. These mean values and the mean values for the parameters of digestion for each diet were then used to determine correlation coefficients.

During continuous intravenous infusion of [^{14}C]glucose after a priming injection the specific radioactivity of the plasma glucose 60–240 min after the start of the infusion was relatively constant. Glucose entry rates were therefore calculated from the following formula:

$$\text{glucose entry rate (mg/min)} = \frac{\text{rate of infusion of } ^{14}\text{C } (\mu\text{Ci/min})}{\text{mean } ^{14}\text{C in plasma glucose after 60–240 min } (\mu\text{Ci/mg})}.$$

III. Results

(*a*) *Plasma Glucose*

The mean plasma glucose concentration varied between 46 and 64 mg/100 ml with the various diets. A lower mean of 39 mg/100 ml was observed in fasted animals. The glucose concentration was significantly correlated with DOM intake ($r = 0{\cdot}51, P < 0{\cdot}05$) though the range in glucose concentrations was relatively small.

(*b*) *Plasma Insulin*

Insulin concentrations on the various diets ranged between 10·0 and 30·0 microunits/ml. The mean insulin concentration was not closely correlated with the

glucose concentration ($r = 0{\cdot}28$, n.s.), but it was correlated with the DOM intake ($r = 0{\cdot}74$, $P < 0{\cdot}001$) (Fig. 1).

The insulin concentration was also correlated ($r = 0{\cdot}74$, $P < 0{\cdot}001$) with the quantity of NAN digested in the intestines (Fig. 1). The relationship was not merely a result of differences in the rate of flow of digesta through the gastrointestinal tract, as the insulin concentration was poorly correlated with the daily flow of digesta out of the abomasum ($r = 0{\cdot}33$, n.s.).

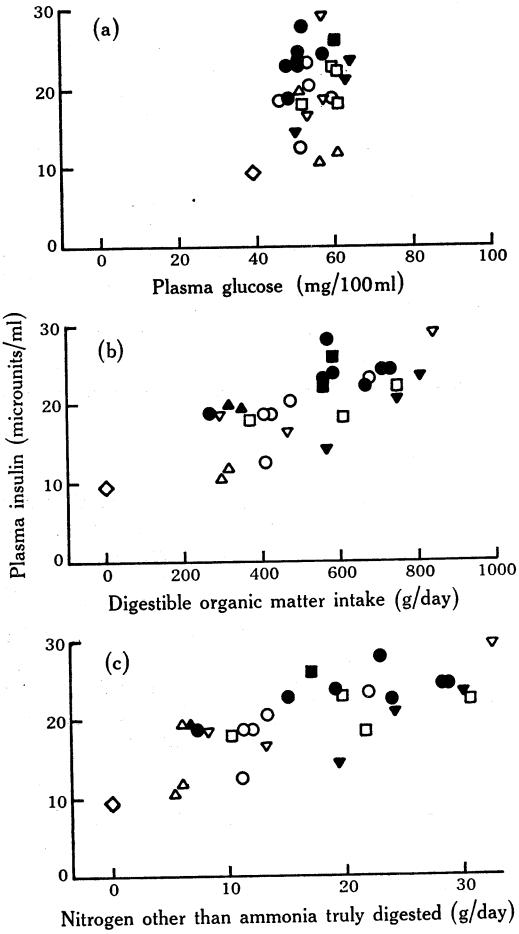

Fig. 1.—Relation of mean plasma insulin concentrations to (*a*) plasma glucose, (*b*) the amount of organic matter digested in the alimentary tract, and (*c*) the amount of nitrogen in forms other than ammonia truly digested in the intestine in groups of sheep fasted (◊) or fed on ryegrass (○), forage oats (●), dried phalaris (□), subterranean clover (▽), Berseem clover (▼), wheaten hay (△), lucerne hay (■), or mixed concentrates (▲).

Despite the significant relationship between insulin and the DOM intake, insulin was not closely related to the total ruminal production of VFA nor to the production of the individual acids (Table 1). Among the VFA, propionate production was most strongly and butyrate production least strongly correlated with the insulin concentration. The correlations were weaker than that between the amount of NAN and insulin (Table 1). The rates for ruminal VFA production were determined for only some of the diets, so correlations of daily DOM intake and NAN with insulin on these diets are also included in Table 1.

The insulin concentration was not significantly correlated with the total concentration of amino acids in plasma, but concentrations of tyrosine, valine,

TABLE 1

RELATION OF MEAN PLASMA INSULIN CONCENTRATIONS TO THE MEAN RATES OF VFA PRODUCTION AND NITROGEN DIGESTION IN SHEEP FED A VARIETY OF DIETS*

Correlation of Plasma Insulin Concentration with:	Correlation Coefficient, r	Significance
Digestible organic matter intake	0·64	$P < 0·001$
Ruminal VFA production		
Total	0·48	$P < 0·05$
(*a*) Acetate	0·45	$P < 0·05$
(*b*) Propionate	0·51	$P < 0·02$
(*c*) Butyrate	0·40	$P < 0·1$
Non-ammonia nitrogen truly digested in intestines	0·69	$P < 0·001$

* The 22 diets included five different ryegrasses, seven forage oats, four phalaris, and six clovers varying in maturity.

TABLE 2

CORRELATION OF MEAN PLASMA INSULIN CONCENTRATION WITH MEAN PLASMA CONCENTRATIONS OF FREE AMINO ACIDS IN SHEEP FASTED OR FED A VARIETY OF DIETS*

Correlation of Plasma Insulin Concentration with:	n	Correlation Coefficient, r	Significance
Non-ammonia nitrogen truly digested in intestines	12	0·82	$P < 0·001$
Total free amino acids	12	0·34	n.s.
Individual amino acids			
Valine	12	0·72	$P < 0·01$
Cystine+methionine	10	0·20	n.s.
Isoleucine	12	0·61	$P < 0·05$
Leucine	12	0·49	n.s.
Phenylalanine	12	0·56	$P < 0·05$
Lysine	12	−0·06	n.s.
Histidine	12	−0·44	n.s.
Arginine	12	0·41	n.s.
Glycine	12	−0·39	n.s.
Alanine	12	0·49	n.s.
Tyrosine	12	0·72	$P < 0·01$
Threonine	8†	0·20	n.s.

* The rations used included four clovers and three phalaris diets for which mean values on three or more sheep were available and four ryegrass diets for which values on only one sheep were available in each case.

† No values for threonine were obtained for the four ryegrass diets.

isoleucine, and phenylalanine were significantly correlated with that of insulin (Table 2). None of the correlations between insulin and the individual free amino

acids was as great as that between insulin concentration and NAN on these diets ($r = 0{\cdot}82$, $P < 0{\cdot}001$).

Mean insulin concentrations in sheep fed ryegrass diets at 90% of their *ad libitum* intake, at half this amount, or after fasting for 3 days were closely correlated with glucose entry rates (Fig. 2). Both the insulin concentration and glucose entry

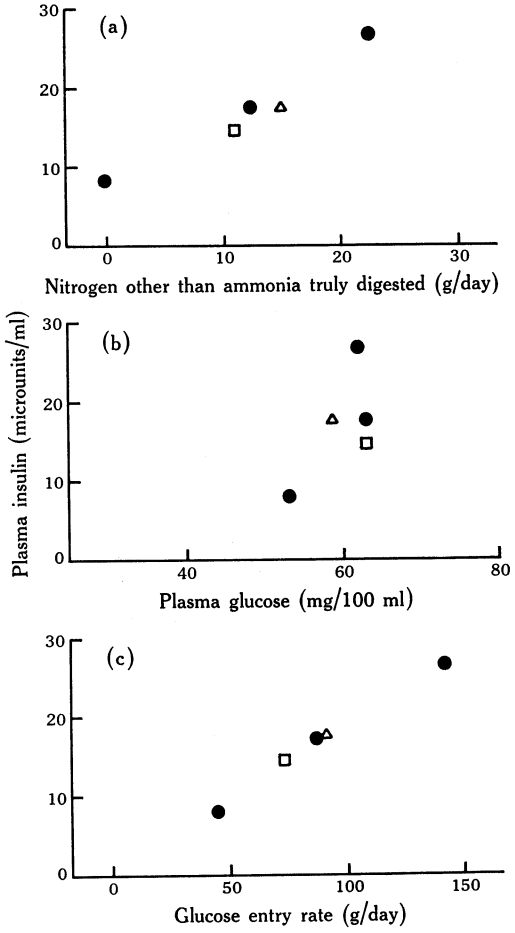

Fig. 2.—Relation of mean plasma insulin concentration to (*a*) the amount of nitrogen in forms other than ammonia digested in the intestines, (*b*) the mean plasma glucose, and (*c*) the mean glucose entry rate in a group of three sheep fed on early cut ryegrass at near *ad libitum*, half this amount, or fasted (●), and in groups of three sheep fed *ad libitum* mid-season (△) or late-cut (□) ryegrass.

rate were closely related to the amount of NAN. The plasma insulin concentration was not related to the plasma glucose concentration (Fig. 2). In the 15 individual experiments, glucose entry rate was significantly correlated with the plasma insulin concentration ($r = 0{\cdot}76$, $P < 0{\cdot}001$), but not with the plasma glucose concentration ($r = 0{\cdot}41$). Plasma concentrations of insulin and glucose were not significantly correlated ($r = 0{\cdot}25$).

(*c*) *Plasma Growth Hormone*

The mean growth hormone concentrations varied from values less than 1·0 ng/ml up to 5·0 ng/ml on the different diets. These values were negatively

correlated with the daily DOM intake ($r = -0{\cdot}62$, $P < 0{\cdot}01$), NAN ($r = -0{\cdot}63$, $P < 0{\cdot}01$), and with the insulin concentration ($r = -0{\cdot}71$, $P < 0{\cdot}01$) (Fig. 3).

The growth hormone concentration was not significantly correlated with the total concentration of free amino acids in plasma ($r = -0{\cdot}39$) but was significantly correlated with the plasma concentrations of the individual amino acids valine ($r = -0{\cdot}63$), alanine ($r = -0{\cdot}62$), tyrosine ($r = -0{\cdot}62$), and threonine ($r = -0{\cdot}65$). These correlations were similar to that with NAN on these diets ($r = -0{\cdot}59$). Correlations with other individual amino acids were not significant.

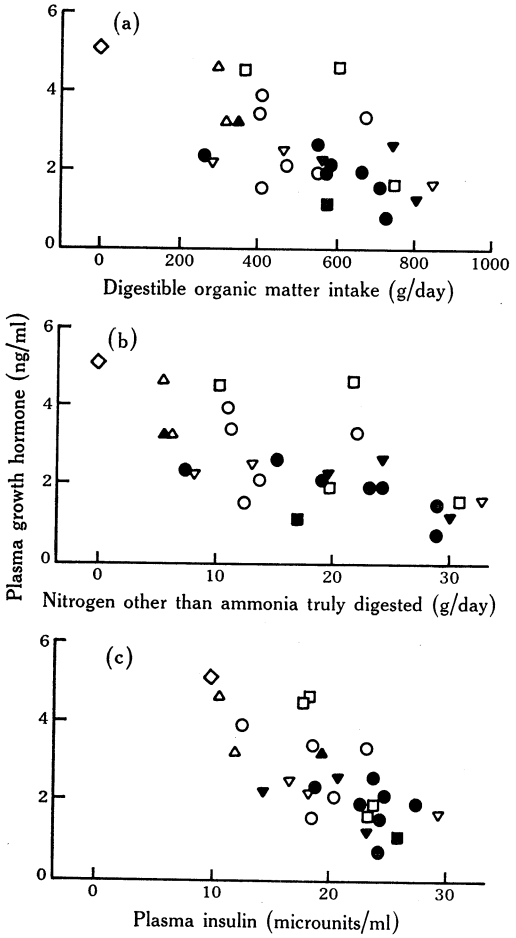

Fig. 3.—Relation of mean plasma growth hormone levels to (*a*) the amount of organic matter digested in the alimentary tract, (*b*) the amount of nitrogen digested in the intestine, and (*c*) the mean plasma insulin concentration in groups of sheep fasted or fed on a variety of forage and roughage diets (symbols as in Fig. 1).

IV. Discussion

The present experiments demonstrate that under the varied dietary conditions studied the plasma concentrations of insulin and growth hormone are significantly correlated with the intake of digestible nutrients and confirm that in the sheep the metabolic dispostion of absorbed nutrients is hormonally regulated.

One function of the endocrine system is the homeostasis of blood glucose. Some relationship between the plasma glucose concentration and the DOM intake was

demonstrated, but the range of glucose concentrations was very small, excluding those in fasted sheep. Others have demonstrated that the glucose concentration in sheep is related to the glucose entry rate (Annison and White 1961; Bergman 1964; Annison *et al.* 1967). However, our observations on glucose entry rate, though few, suggest that the plasma concentration of glucose is to a large extent independent of the glucose entry rate. This probably applied to all our diets because the range of entry rates on ryegrass diets was probably almost as great as that on any of the diets used.

The clear association between the rate of glucose entry and the plasma concentration of insulin confirms that insulin, despite its lack of correlation with the glucose concentration, has an important role in glucose homeostasis. This conclusion is supported by the association of both glucose entry rate and the concentration of insulin in plasma with the rate of intestinal protein digestion (NAN). Furthermore, though the concentration of growth hormone in plasma was not measured during the studies in which glucose entry was determined, the significant inverse correlation between growth hormone and insulin concentrations in plasma in the main study suggests that growth hormone is also concerned in glucose homeostasis. These experiments also suggest that the rate of production of digesta influences the plasma concentrations of insulin and growth hormone.

As the plasma concentrations of glucose fell in a narrow range, the plasma insulin concentration could not be shown to depend on plasma glucose concentrations, even though there is evidence that increases in the glucose concentration increase insulin secretion in sheep (Boda 1964; Bassett and Wallace 1967; Manns and Boda 1967; Horino *et al.* 1968). Since infusion of either propionic or butyric acids into the peripheral or portal circulation also stimulates insulin secretion in sheep (Manns and Boda 1967; Manns, Boda, and Willes 1967; Horino *et al.* 1968), it has been postulated that these acids are important stimuli to insulin secretion. However, the relatively poor correlation between the production of propionate in the rumen and the insulin concentration in plasma and the virtual absence of correlation between butyrate production and insulin concentration in the present experiments suggest that the VFA are probably not the major stimuli to insulin secretion in sheep, at least under conditions of frequent feeding.

In view of the weak correlations between ruminal VFA production and the plasma insulin concentration, the stronger correlation of the insulin concentration with the amount of protein digested in the intestines (NAN) is striking. From the magnitude of this correlation, it appears that the rate of protein digestion in the intestine may be an important determinant of the plasma concentration of insulin in sheep. Protein digestion can stimulate insulin secretion in man (Floyd *et al.* 1966*a*), and many of the amino acids, when infused intravenously, can also stimulate insulin secretion (Floyd *et al.* 1966*b*). However, although arginine can stimulate insulin secretion in sheep (Machlin *et al.* 1968) there is little evidence in the present study that the insulin concentration is closely related to the total concentration of free amino acids in the plasma, or to the concentrations of individual amino acids other than tyrosine and valine.

The insulin secretory response to both glucose (McIntyre, Holdsworth, and Turner 1964) and arginine (Dupré *et al.* 1968) is greater when they are administered

orally rather than intravenously. Further, the gastrointestinal hormones, secretin, pancreozymin, and an unidentified hormone immunologically similar to glucagon, can all stimulate insulin secretion (Unger *et al.* 1967; Unger *et al.* 1968), and may potentiate the insulin secretory response to glucose or amino acids (Ohneda *et al.* 1968; Kraegen *et al.* 1970). Secretion of the gastrointestinal hormones is therefore intimately involved in the insulin secretory response to feeding. The high correlation between the amount of protein digested in the intestines and the insulin concentration in plasma suggests that the gastrointestinal hormones may play a major role in regulating the secretion of insulin in sheep.

Evidence that the plasma concentration of growth hormone was inversely related to the level of nutrition was somewhat unexpected in view of earlier findings that growth hormone concentrations in sheep were not increased by fasting (Machlin *et al.* 1968; Wallace and Bassett 1970). However, Roth *et al.* (1963) demonstrated the existence in man of a negative feedback system between glucose utilization and growth hormone secretion and this has been amply confirmed by subsequent work (Baylis *et al.* 1968). Since the insulin concentration of sheep is related to the rate of glucose turnover, the negative correlation between insulin and growth hormone concentrations may reflect the existence of a similar negative feedback system between glucose utilization and growth hormone secretion in the sheep. Alternatively, digestion in the intestines may, in some way, influence growth hormone secretion.

While this study of relationships between plasma metabolite and hormone concentrations and parameters of digestion can only yield tentative conclusions, it is evident from the results that both insulin and growth hormone are intimately concerned in regulating the metabolism of absorbed nutrients and that the secretion of both hormones is greatly dependent on nutrient intake.

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