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<td>KAMALU, T.N.</td>
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FREE COMMUNICATION

EFFECTS OF FEEDING, FASTING, INTRAVENOUS INFUSION OF
OLEIC ACID OR CASEIN HYDROLYSATE ON PLASMA GLUCAGON
LEVELS IN SHEEP

Dear Colleague,

We are pleased to advise you that the Scientific Program Committee of the
XI INTERNATIONAL CONGRESS OF NUTRITION has approved the above-mentioned
paper for presentation in a Free Communications session.

Details of day, time and room number will be published in the General
Program of the Congress which you will receive together with the rest
of your documentation upon registration at the Congress center in Rio
de Janeiro.

If you have not already done so, please ensure that you are registered as
an Active Member of the Congress so that your name may appear in the Book
of Members, and your abstract in the Book of Abstracts.

We require a final text of your paper by June 30th typed in single-spacing
on plain white paper for publication in the Proceedings of the XI Interna-
tional Congress of Nutrition. Please send us two copies of each final
text, one original and one photocopy.

We look forward to seeing you at the Congress in August, and attach
an extra sheet of information to guide you.

Yours sincerely,

Prof. Nabuco Lopes
Vice-President
June 21, 78

Dear Sir,

Please find enclosed one original and one copy of the final text of my paper entitled:

"Affects of feeding, fasting, intravenous infusion of citric acid or tissue hydrolyzate on plasma phosphate levels in sheep".

Yours sincerely,

[Signature]

Dr. T. J. Kaufman
EFFECTS OF FEEDING, FASTING, INTRAVENOUS INFUSION OF OLEIC ACID OR CASEIN HYDROLYSATE ON PLASMA GLUCAGON LEVELS IN SHEEP
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Department Of Veterinary Anatomy & Physiology
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And
Allen H. Trenkle
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Iowa state University, Ames, Iowa.

TREATMENT OF THE DUMPING SYNDROME WITH THICKENED "SLOW RELEASE" MEALS. A.R. Leedes (I.), D.J.A. Jenkins (2), C. Pett (3), D.R.S. Ralphs (3), T. Mein Research Fellow, Dept. of Nutrition, Queen Elizabeth College, London, W8, 2. Dept. Gastroenterology, Central Middlesex Hospital, London NW10, 3. Dept. Surgery, The Middlesex Hospital, London, W1, United Kingdom. Pectin, a partially methylolated polymer of galacturonuronic acid derived from citrus peel, has been shown to decrease post-prandial glycaemia in normal subjects, when mixed with carbohydrate meals. Since there is often an exaggerated glycaemia and rebound hypoglycaemia in patients who develop the dumping syndrome after gastric surgery this group was studied. It was shown that:

- The addition of pectin to glucose test meals caused:
  - a reduction of blood glucose levels during the first hour of the test,
  - and increased levels at 1 1/2 and 2 hours, i.e. rebound hypoglycaemia
    was prevented.
  - a reduction of plasma volume changes (calculated from haematocrit
    changes).
  - abolition or reduction in severity of symptoms during the test.
  - slowing of rapid gastric emptying (assessed by T-cameras method).
- Treatment of 8 patients with 7g Pectin daily for up to 6 weeks did not
  significantly decrease symptom frequency.
- The addition of Pectin to the test meals resulted in a 1000-fold increase
  in meal viscosity and the results suggest that regular administration
  would decrease symptom frequency. However, the negative findings of the
  clinical trial may have been due to the choice of a dose which did not
  cause a sufficient rise of meal viscosity.

The Hercules Powder Co. Ltd. kindly supplied the Pectin.

* * *

EFFECTS OF FEEDING, FASTING, INTRAVENOUS INFUSION OF OLEIC ACID OR CASEIN HYDROLYZATE ON PLASMA GLUCAGON LEVELS IN SHEEP, T. N. Ramulu
and Adj. Trenchle, University of Nigeria, Nsukka, Nigeria and Iowa State University, Ames, Iowa.

Experiments were carried out to study some aspects of the nutritional regulation of plasma glucagon concentrations in sheep and their relation to plasma levels of insulin. Radioimmunoassay method was used to measure changes in plasma levels of glucagon and insulin in 35-40 kg crossbred lambs following feeding, fasting intravenous infusion of oleic acid or casein hydrolysate. Neither feeding nor 24 hrs. fast had any significant effect on plasma glucagon levels. Casein hydrolysate also had no appreciable effect on plasma glucagon levels. However, oleic acid had a depressing effect on plasma glucagon concentrations. Plasma insulin concentrations were elevated after feeding and declined as fasting progressed. Intravenous injection of oleic acid or casein hydrolysate increased insulin concentrations. These results indicate that the factors studied seem to have greater influence on plasma insulin than on plasma glucagon levels. However, since glucagon may play a role in regulating insulin secretion, it is suggested that a biologically significant amount of glucagon secretion might have occurred in the pancreas without a measurable change in glucagon concentrations in peripheral blood.
INTRODUCTION

In ruminants carbohydrates are fermented to short chain fatty acids in the rumen and there is little absorption of glucose as such (Hugate, 1966). Hence ruminants have low blood glucose which remains low even after feeding. The glucose requirements of these species are not by gluconeogenesis (Katz and Bergman, 1969). Thus changes in blood glucose level which regulate pancreatic endocrine secretions in monogastrics seem to have a diminished role in ruminants. Indeed, there is evidence that glucose is not important in regulating plasma glucagon levels in sheep (Basset, 1972; Arcus et al, 1976). Several studies have revealed that the short chain fatty acids are the principal regulators of insulin and glucagon secretion in ruminants (Manns and Boda, 1967; Harano et al., 1968; Trunkle, 1970; Komaru et al.; 1970; Bassette, 1972; Arcus et al., 1976). While the regulation of insulin secretion in ruminants by various metabolic factors is well established, little attention has been given to the regulation of glucagon.

The studies reported here were carried out to examine changes in plasma glucagon concentrations in sheep and their relation to level of plasma insulin. The effects of feeding, fasting, long chain fatty acid (oleic acid) and amino acids (casein hydrolysate) were investigated.

MATERIALS AND METHODS

Animals: All the animals used in these experiments were crossbred wethers weighing between 35kg to 40kg. They were fed 0.9kg per animal per day of a diet containing 35% cracked shelled corn, 10% ground corn cobs, 40% ground alfalfa hay, 6% soybean meal, 8% molasses, and 1% trace mineralized salt. Except where otherwise indicated, the animals were fed twice daily.

Blood samples were obtained in heparinized tubes. As soon as obtained, the blood samples were chilled in ice, centrifuged at 4°C and plasma frozen until assayed.
Effect of Feeding and Fasting: The animals were fed once a day and the effects of feeding and 24 hours fasting on plasma glucagon levels studied. Catheters were placed in the jugular veins of four lambs and blood samples obtained at -2, -1, 6, 1, 5, 3, 5, 7, 12, and 24 hours after feeding. The blood samples were analyzed for plasma glucagon, insulin, and free fatty acids (FFA), and glucose. Plasma glucagon and insulin concentrations were measured by the dextrans and protein coated charcoal radioimmunoassay method described previously for insulin (Trenkle, 1970) and adopted for glucagon (Kamal, 1970). Plasma glucose was measured by an automated modification of the method of Hoffman (1937). Plasma FFA was determined by the method of Ko and Royer (1967).

Oleic Acid: Two lambs were fasted and injected with 20ml of an emulsion of 1.5ml oleic acid in 30ml of plasma, the pH was adjusted to 7.4. The plasma used to prepare the emulsion had previously been obtained from the respective animals. Blood samples were obtained by means of catheters in the jugular vein at -30,-15,0,15,30,45,60,90, and 120 mins after injection. The samples were analyzed for glucagon, insulin, glucose and FFA by the methods indicated above.

Casein Hydrolysate: Catheters were placed into both the right and left jugular veins of lambs. One catheter was used for infusion and the other for withdrawing blood. A 50% solution (w/v) casein hydrolysate was prepared by dissolving acid hydrolyzed casein (General Biochemicals, Lot No. 83027) in 1.0N NaOH and neutralizing to pH 7.4 with HCl. Distilled water was added to make up to volume. After overnight fast, the animals were infused with 0.15N NaCl for 45mins at the rate of 3ml per min with a peristaltic infusion pump. After 45mins, the solution containing hydrolyzed casein was infused for 60mins. Blood samples were obtained at 15mins intervals during and after the infusion. The samples were analyzed for glucagon, glucose, insulin, and alpha amino nitrogen (AAN). Plasma glucagon, insulin and glucose were measured by the methods indicated above. Plasma AAN was measured by the method of Spackman et al. (1958) modified for determination with the Technical Auto Analyzer.

The data were treated statistically by analysis of variance and duncan’s multiple range test (Steel and Torrie, 1963).
RESULTS

Effect of feeding and fasting. The effect of feeding and fasting on plasma hormone and metabolites concentrations are shown in Fig. 1. Plasma glucagon concentrations showed no significant changes (p>0.05) before or at any time after feeding during the period studied. Mean insulin levels increased after feeding and then declined as fasting progress. The increase was statistically significant only at 1.5 hours after feeding (p<0.05). There was little change in the mean plasma glucose levels after feeding. However, plasma FFA levels declined after feeding to reach their lowest concentrations between 3 and 5 hours postprandia, and then to rise to significantly higher levels at 24 hours (p<0.05).

Oleic Acid. Figure 2, shows that intravenous injections of emulsified oleic acid elevated plasma FFA concentrations in the two lambs (p<0.01). Glucose concentration increased slightly in one animal but not in the other. Plasma glucagon concentrations fell in both animals during the period of 45 minutes following oleic acid injection. However, the magnitude of change was not statistically significant (p>0.05). Insulin concentrations, on the other hand were elevated 3 fold (p<0.01) by 15 minutes post injection.

Casein Hydrolysate. As casein hydrolysate was infused, plasma AAN concentration gradually increased (p<0.01) until the termination of the infusion (Figure 3). Glucagon levels were not appreciably affected by the infusion of casein hydrolysate. The changes in glucose and insulin concentrations were significant (p<0.05) and paralleled the changes in AAN. Insulin levels, however, continued to rise for 30 minutes after the infusion of casein hydrolysate had been stopped.

DISCUSSION

The observed effects of feeding on insulin, glucose and FFA levels confirm previous work by Trenkle (1970). However, the failure of glucagon concentrations to rise after feeding is at variance with the observations of others who showed increases in glucagon levels following feeding (Boies; 1972; Vance et al., 1969). The reason for the discrepancy is not clear. It is known, from the studies of Vance et al. (1968) in human subjects, that glucagon response to feeding could be a statistically nonsignificant transient rise. It is also known that there are two sources of plasma immunoreactive glucagon—the pancreas and the gut, and that plasma glucagon may rise while plasma...
pancreatic glucagon declines following intraduodenal loading of glucose (Unger et al., 1968; Valverde et al., 1968). Thus changes in the secretion of glucagon that could have biological effects, such as stimulation of insulin release, can occur without causing a detectable change in assayable peripheral immunoreactive glucagon (Ketterer et al., 1967).

Reports in the literature on the changes in plasma glucagon levels during fasting have been controversial. Unger et al. (1963) and Angilhar-Parada et al. (1969) reported elevation in plasma glucagon levels during starvation in man. This was not confirmed by Vanee et al. (1968) in either human subjects or dogs. On the other hand they found glucagon levels to fall or remain unchanged during starvation. In ruminants, Bassett (1972) indicated little variation in fasting glucagon levels from day to day, with steady decline during prolonged starvation. Our observation of little change in plasma glucagon levels during a 24 hours fast is in agreement with the findings of Vanee et al (1968) and Bassett (1972).

The observed effect of oleic acid on glucagon secretion made in the present study is in agreement with the reports of Madison et al (1968), Pi-Sanya et al (1969) and Crespin et al (1969). It is thought that elevated plasma FFA concentrations exert a feedback inhibition effect on glucagon secretion while stimulating insulin secretion. Support for the feedback mechanism is given by the work of Luyckx and Leeberuve (1970) who found that the depression of plasma FFA levels with nicotinic acid stimulated glucagon secretion but that triglyceride infusion inhibited it. This concept of FFA control of glucagon secretion does not conflict with, and may probably explain, the observations of declining or non-elevated glucagon levels during fasting.

Infusion or ingestion of amino acids and proteins has been found to stimulate insulin and glucagon secretion (Floyd et al., 1966; Fajans et al., 1967; Pek et al., 1968, 1969). Differences in response to various individual amino acids or mixture of amino acids exist. Lecine was found to have no effect on glucagon secretion (Pek et al., 1969) and arginine was found to be about five times more potent in stimulating glucagon secretion than a mixture of the ten essential amino acids (Pek et al., 1968). Insulin response differs from glucagon response in that a mixture of the ten essential amino acids stimulates more insulin secretion than arginine (Floyd et al., 1966; Fajans et al., 1967). Our experience with the use of casein hydrolysate in sheep is that a very high dose is needed to produce effect. The infusion of a 5% casein hydrolysate solution
during a preliminary experiment was without effect even on insulin secretion. Hence the use of a 10% solution in this study. Plasma insulin levels were elevated as a result of the infusion of the 10% casein hydrolysate solution, but we could not demonstrate any effect on glycerone concentrations. It is worth mentioning that in studies with auto-transplanted pancreas in sheep, Arcus et al. (1976) observed increases in plasma glucagon levels by injecting arginine directly to transplant. There is, however, considerable doubt as to the role of amino acids in the physiological regulation of pancreatic hormones in eukaryotes since there is little increase in concentrations of plasma amino acids in these species after feeding (Thews et al. 1966).

REFERENCES

Figure 3. Effect of infusing 10 percent protein hydrolysate solution on plasma alpha amino nitrogen (AAN), glucose, insulin and glucagon concentrations (Mean ± S.E.M.)
Figure 2. Effect of injecting plasma emulsion of oleic acid on plasma free fatty acid (FFA), glucose, insulin and glucagon concentration.