THE EFFECTS OF EUGLYCEMIC INSULIN CLAMP ON THE METABOLISM OF 3-HYDROXYBUTYRATE AND PLASMA FREE FATTY ACIDS BY THE HIND LIMB OF LACTATING SHEEP

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(Received 3 March 1988; accepted for publication 20 May 1988)

ABSTRACT

The effects of exogenous insulin, with euglycemia, on the concentration differences, extraction ratio and uptake of 3- hydroxybutyrate and plasma free fatty acids by the lactating sheep hind limb were studied.

Generally, the results showed that with increasing plasma insulin levels, while maintaining euglycemia, the concentrations, arterio-venous concentration differences, extraction ratio and uptake of 3-hydroxybutyrate and plasma free fatty acids were significantly ($P < 0.05$) reduced. Positive but significant ($P < 0.01$) correlation was established between arterial concentration of 3-hydroxybutyrate and plasma free fatty acids and their uptake. A similar relationship was also obtained between the arterial concentrations of 3-hydroxybutyrate and plasma free fatty acids.

Key Words: Euglycemic insulin clamp, Metabolism, 3- hydroxybutyrate plasma free fatty acids, lactating sheep.

INTRODUCTION

Euglycemic insulin clamp technique is used essentially to quantify tissue sensitivity to exogenous insulin in conjunction with labelled glucose to define the site of insulin resistance (Defronzo *et al.*, 1979). This technique has been used mostly for human studies (Kolterman *et al.*, 1980; Rizza *et al.*, 1981). Recently, however, the technique was extended to ruminant species (Janes *et al.*, 1985; Weeks *et al.*, 1983). The effects of euglycemic insulin clamp on blood metabolites other than glucose, have not been fully investigated. Effects of insulin infusion or injection without maintenance of euglycemia upon circulating metabolites, have been extensively studied. There is evidence (Radloff and Schultz, 1966) that in ruminants insulin caused a depression of plasma free fatty acids (FFA), followed by a sharp rise. In normal rats, a lipolytic effect of insulin was observed, even when the blood glucose was maintained above 200mg/100ml (Kovacev and Scow, 1966). Studies have also shown that injection of insulin increased the plasma FFA concentrations in dog (Armstrong *et al.*, 1961) and in man (Week *et al.*, 1961).

In ruminants insulin was shown to have little effect on blood ketone concentrations (Kronfeld 1969; Radloff and Schultz, 1966). However, insulin has been reported to en-

hance utilization of ketone bodies in rats (Beatty *et al.*, 1964). Prior *et al.*, (1984) reported positive uptake of acetate, propionate and butyrate, and increased extraction ratio of glucose, but a decreased extraction ratio of L-lactate, by the hind half of beef steers, following infusion of insulin. Studies, involving the measurements of metabolite uptake by the sheep hind limb, showed that FFA are the main metabolites at rest and during exercise, while 3-hydroxybutyrate (3-0HB) and acetoacetate are major nutrients in starved animals (Jarrett *et al.*, 1976). Similar studies (Pethick *et al.*, 1983) using pregnant ewes, showed that both the hind limb muscles utilized and produced non- esterified fatty acids.

In man, it has been shown that muscle would account for 85% glucose removal in euglycemic clamp and that raised insulin levels stimulated glucose oxidation (Defronzo *et al.*, 1981) Theoretically, such effect on glucose metabolism may be expected to 'spare' other high energy sources like 3-0HB and FFA for use by other tissues of the body, where insulin may be less sensitive. The object of the present study, therefore, was to determine the effects of insulin without hypoglycemia on the concentrations, arterio-venous (A-V) concentration differences, extraction ratio and uptake of 3-0HB and plasma FFA by the hind muscle of lactating sheep.

MINERALS AND METHODS

Four lactating ewes (mean weight 52.1 ± 3.8 kg), in mid-lactation (mean milk yield 1.98 ± 0.34 kg/day) were used in the experiment (± standard error). They were housed individually in metabolism crates. The animals, were hand milked at 0800 and 1600h. each day and were fed *ad libitum* on hay and a high-protein barley based pelleted concentrate; the estimated dry matter intake per animal was 1.68 ± 0.24 kg/day, salt licks and water were continuously available.

Prior to experimentation, polethylene or silastic catheters (0.03 in inner diameter, 0.065 in, outer diameter Dewcorning Corporation Medical Products Midland Michigan, U.S.A. 48640) had been introduced under general anaesthesia into jugular vein, carotid artery and deep femoral vein via the recurrent tarsal vein (Oddy *et al.*, 1981) of each animal. The catheters were filled with sterile heparinized saline (250 i.u./ml) and their patency checked daily.

The euglycemic clamp procedure adopted in this study was as outlined (Weekes *et al.*, 1983; Janes *et al.*, 1985). Sequential insulin infusions at the rates of 0.33, 1.0, 3.0 and 15.0 UU/min. per kg. live weight were administered to the animals through the jugular vein over periods of 90 minutes. Euglycemic condition was maintained by monitoring the plasma glucose level at 10 minutes intervals, throughout the experiment, and by adjusting the glucose infusion rate. Two experiments at mid- lactation were carried out, with 8h. duration each. In both experiments the plasma glucose level was maintained at 68.66 ± 0.75mg/100ml., a value that was highly comparable with the pre-insulin infusion plasma glucose value of 67.49 ± 0.41mg/100ml. Blood flow measurements (Oddy *et al.*, 1981) and Brown *et al.*, 1982) were performed on the standing restive animals 24h. after each euglycemic clamp. And the calculation of blood flow was also as outlined (Oddy *et al.*, 1981). Blood for 3-0HB and plasma FFA determination was collected every two hours from carotid artery and femoral vein. In addition, pre and post (24h,) infusion blood samples were also taken. Plasma glucose concentration was estimated as described (Janes *et al.*, 1985) while plasma insulin concentration was determined immunologically (Fuller *et al.*, 1977). The blood filtrate after deproteinisation (Somogyi, 1945) was used in determining 3- 0HB concentrations (Wil-

liamson *et al.*, 1962). The FFA were extracted from the plasma according to Dole and Meinertz, (1960) and measured.

Extraction ratio was calculated as the arterio-venous (A-V) concentration difference divided by arterial concentration, times 100. Net uptake or release by the hind limb of the ewes was calculated as blood flow or plasma flow rate times the arterior-venous (A-V) concentration difference. Linear correlation was performed to examine associations between arterial concentrations and uptake of 3-0HB and plasma FFA by the sheep hind limb. Similar relationship was also determined between arterial concentrations of 3-0HB and plasma FFA. Results were evaluated by analysis of variance and the significance of differences between different means was tested by studentized range test (Snedecor and Cochrane, 1967).

RESULTS

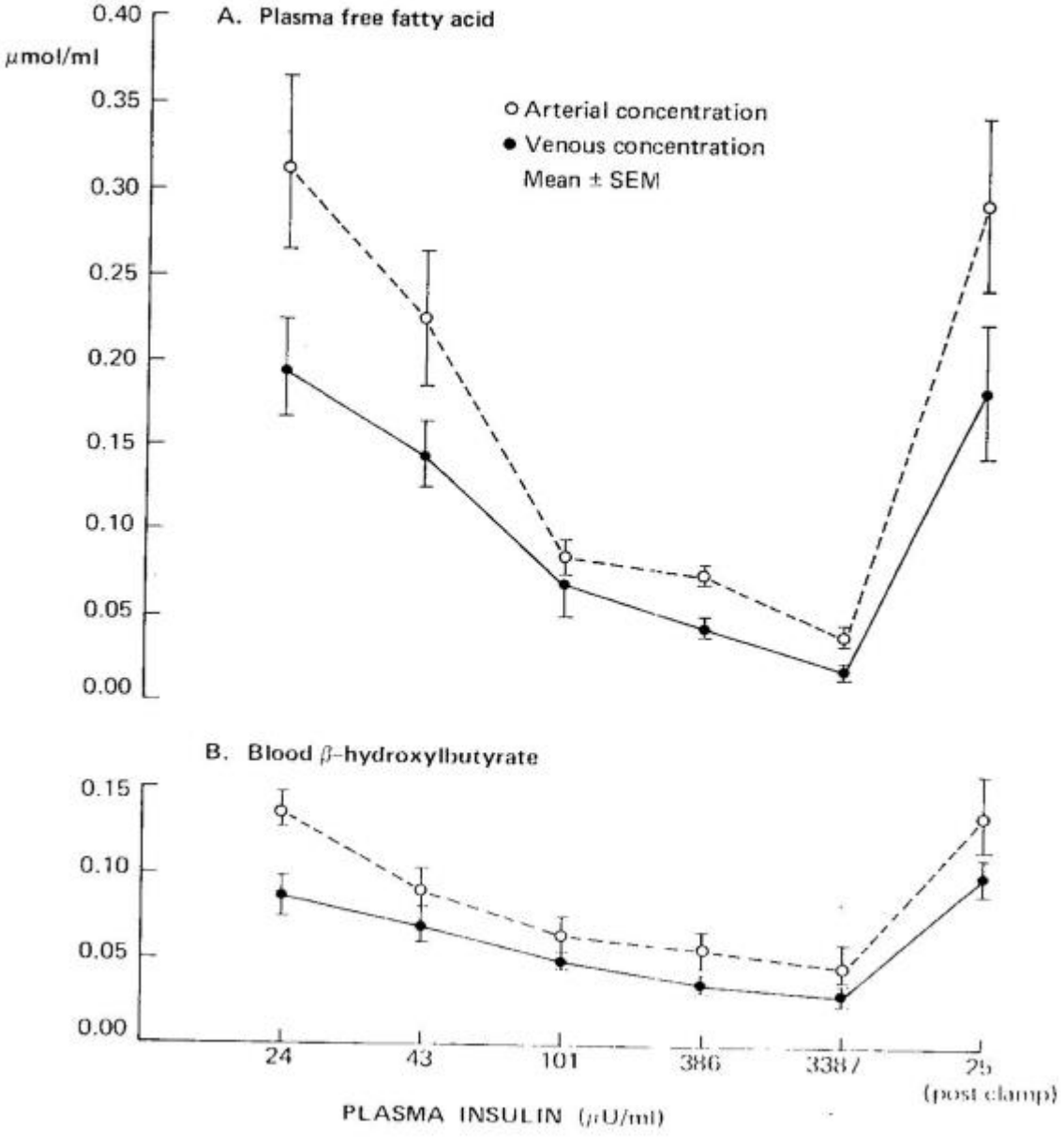
There was no significant difference between the mean values obtained in the first and second experiment; hence the two values were pooled and used in subsequent calculations. Results obtained in the present study are presented in Figure 1. The trends depicted a decrease ($P<0.01$) with increasing plasma insulin levels on both the 3-0HB and plasma FFA concentrations. Mean basal (pre-insulin infusion) values and those obtained at the lowest rate of insulin infusion were similar just as the difference between the basal and post-insulin infusion values were not significant ($P>0.05$). Basal arterial 3-0HB and plasma FFA concentrations averaged 0.136 and 0.313μm/ml, respectively, which corresponded with the mean plasma insulin value of 24μm/ml., but at the highest level of plasma insulin value of 3398μm/ul., the 30HB and plasma FFA decreased by over 65 and 87% respectively. The venous concentrations were also depressed during insulin infusion.

The A-V concentration differences also decreased ($P<0.01$) during the insulin treatment. The extraction ratio of 3-0HB was not significantly affected ($P>0.05$) by the insulin treatment. There was, however, a significant ($P<0.05$) effect on the extraction ratio of plasma FFA. The highest extraction ratio of plasma FFA of 51% was obtained at the highest level of insulin and it was about 38% higher than the basal extraction ratio. The uptake of both 3-0HB and plasma FFA by the sheep hind limb was significantly ($P<0.05$) affected by the insulin treatment. Positive but significant ($P<0.01$) correlations were established between the arterial 3-0HB concentration and its uptake ($r = 0.64$) and arterial concentrations of 3-0HB and plasma FFA ($r = 0.98$).

DISCUSSION

The results of this study showed that there were significant reduction in 3-0HB and plasma FFA concentrations, when the level of plasma insulin was increased. Insulin has previously been shown to have inhibitory effect upon ketone body production in the sheep liver (Brockman and Laarveld, 1985). However, in ruminants, it is not only the liver, which may serve as a site of significant ketone body synthesis but also the rumen epithelium has been shown to metabolise butyrate, a product of rumen fermentation, to yield 3-0HB (Leng and West, 1969). Whilst the literature contains no reference to an antiketogenic effect of insulin upon the rumen epithelium, it remains a matter of conjecture whether the depression of 30 HB evoked by insulin in this experiment resulted from its action on both the liver and the rumen epithelium. Nevertheless the close ($P<0.01$) parallel between the circulating levels of FFA and 3-0HB in this work and the knowledge that FFA are the hepatic substrates for ketone body synthesis (Katz and

Fig. 1: Effect of Eugylycemic Clamp on Plasma Free Fatty Acid (A) and Blood βHydroxylbutyrate (B) Concentration of Lactating Sheep



Bergman, 1969), suggest that the depression in 3-0HB may have been a consequence of substrate limitation resulting from depressed lipolysis.

It seems from the present study, that under euglycemic condition, with high levels of plasma insulin, the sheep hind limb maintained levels of 3-0HB and plasma FFA uptake which correspond with their concentrations. This is in agreement with the concept that the utilization of ketone bodies is related to the quantity of circulating concentration (Krebs *et al*, 1970). Hence, the present studies showed that uptake of 3-0HB and FFA by the sheep hind limb was decreasing significantly ($P < 0.05$) with increasing levels of insulin. Since results obtained showed positive significant correlation between the arterial concentrations of these metabolites and their uptake, the observed decrease in the uptake of these metabolites here may be related to the effect of insulin infusion on the concentration of these metabolite and not as a result of 'sparing' these energy substrates, because of high utilization of glucose by muscle during euglycemic clamp (Defronzo *et al*., 1981). It is, however, expected that with high utilization of glucose by muscle during euglycemic clamp, other energy sources like 3-0HB and plasma FFA would be "spared" for other productive purposes. But this was not so here. Lactation and catecholamine are known to suppress the antilipolytic effect of insulin in order to ensure adequate plasma FFA supply for milk synthesis (Merz Van den Bergh, 1977). Our results showed that at high levels of exogenous insulin, such mechanism may be suppressed and may therefore affect milk production.

However, Laarveld *et al*, (1985) reported no change in the extraction ratio of triglyceride by the mammary gland of the sheep during insulin infusion. The significant ($P < 0.05$) change observed here in the extraction ratio and uptake of plasma FFA by the sheep hind limb, may indicate differences in the mechanism of tryglyceride and plasma FFA uptake by different tissues in the body of the animal.

It is concluded that, while providing a useful research tool in the study of glucose utilization and tissue resistance of insulin, euglycemic clamp with high levels of insulin infusion may affect the quantity of 3-0HB and plasma FFA that are available for tissue utilization through its effects on the concentrations of these metabolites.

ACKNOWLEDGMENT

One of the authors (A.I.A.O.) is grateful to the Royal Society for the award of a Developing Country Fellowship and the University of Ibadan for Study Leave. He is also grateful to Professor D.G. Armstrong for the facilities provided.

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