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Glucagon Administration on Circulatory Metabolites in Induced Hypoinsulinemic Dwarf Goats*

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Abstract.- The goats were rendered diabetic with two consecutive administrations of 33 and 44mg/kg body weight streptozotocin for about 100 days. Glucagon (5µg/kg body weight) was administered on 90th day following induction of diabetes. Hyperglycemia of about 34% ($P<0.05$) was observed at 30 minutes after hormone administration. Likewise significant ($P< 0.05$) hyperaminoacidemia was observed after 30 minutes, however, it remained elevated upto 2 hours following hormone infusion. Glucagon administration caused marked elevation in palmitic acid, myristic acid, oleic acid and linoleic acid. Most of the volatile fatty acids were found to be enhanced after hormone treatment.

Key words: Free amino acids; free fatty acids; goat; glucose; hypoinsulinemic; long chain fatty acids, streptozotocin; volatile fatty acids.

INTRODUCTION

Glucagon consistently induced plasma glucose concentration in dose dependent fashion and improved carbohydrate status (Hippen *et al*., 1999a). Bobe *et al*. (2003c) have demonstrated that both single and multiple injections of glucagon consistently improved carbohydrate status of dairy cows. Glucagon affects enzyme system and the subsequent metabolic pathways widely. The hormone increases gluconeogenesis from propionate and insulin opposes this effect in neonatal bovine liver (Donkin and Armantano, 1994). Similar response of glucagon has been observed in sheep liver caudal lobe (Ali and Jois, 1997) and suckling lamb liver (Savan *et al*., 1986).

Glucagon has an improved role in disposition of amino acids by increasing their inward transport, their degradation and their conversion into glucose (Boden *et al*., 1990). Catabolism of amino acids is enhanced by hyperglucagonemia as the hormone stimulates hepatic gluconeogenesis and reduces plasma amino acid concentration (Boden *et al*., 1983; Gruppuso *et al*., 1983) and liver and muscle free amino acids in the goat (Cheema *et al*., 1993). Glucagon directly or indirectly may mediate amino acid sparing by ruminant liver (Gill *et al*., 1985).

Glucagon decreases the degree of fatty liver in early lactating dairy cows (Hippen *et al*., 1999b; She *et al*., 1999; Bobe *et al*., 2003a). Bobe *et al*. (2003b) also observed decreased plasma lipoprotein fractions of triacylglycerol, phospholipids and cholesterol following glucagon injections in lactating dairy cows. The role of free fatty acid (FFA) as a nutrient secretagogue and a modulator of alpha-cellular glucagon metabolism (Bollheimer *et al*., 2004) and palmitate stimulated glucagon secretion from intact mouse islets in glucose presence (Olofsson *et al*., 2004) have been reported.

The dependence of ruminants on metabolic availability of carbohydrate and the role of glucagon in enhancing its availability through gluconeogensis is well understood. Besides catabolism of amino acids, lipolysis also contributes in gluconeogensis. The molecular basis of elevation of glucagon in circulation and the role of fatty acids contributing in gluconeogensis are sparsely investigated. Since the counter regulatory interaction of glucagon and insulin in intact state depresses the direct effect of glucagon, any study involving the absence or extremely reduced interference of insulin obtained experimentally by destroying the pancreatic ß-cell *in vivo,* will reveal the role of glucagon more explicitly. The present study thus investigates the role of glucagon on circulatory metabolites such as

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free fatty acids in goats rendered hypoinsulinemic with β-cell cytotoxic streptozotocin.

MATERIALS AND METHODS

Adults male dwarf goats, of about three years of age, were maintained with an organized feeding regime of green fodder as well as concentrate dry ration. Goats were acclimated for seven days before start of experimentation. Goats were rendered diabetic, permanently, with two successive administrations of streptozotocin (STZ, Sigma Chemical Company, USA) at a concentration of 33 and 40mg/ kg body weight in saline citrate buffer (pH 4.3) within four days. In an experiment, the goats were administered with glucagon (5 µg/kg body weight) intravenously and repeated blood sampling was obtained just before and 30, 60, 90 and 120 minutes post glucagon treatments.

Glycemia, free amino acids, free fatty acids, long chain fatty acids (LCFAs) and volatile fatty acids (VFAs) were analyzed in sera before and after following hormonal administration in hypoinsulinemic goats. Blood glucose was assayed with glucose oxidase (Barham and Trinder, 1972) and commercial kits (Randox Laboratories Ltd.) were used. A method of Goodwin (1968) was employed for the analysis of plasma free amino acids. The method of Falholt *et al*. (1973) using copper soap formation was used for total plasma free fatty acids (FFAs). For LCFAs plasma FFAs, were extracted from plasma by the method of Falholt *et al.* (1973) and esterified with boron triflouride (Morrison and Smith, 1964) and further extracted with benzene. Volatile fatty acids (VFAs) extracted in redistilled ethanol were measured according to Remesy and Demigne (1974). The samples and the standard mixture from Supelco, Inc. GC Bulletin 748H was analyzed for these fatty acids on gas chromatograph of Perkin-Elmer model 3920.

RESULTS

Glucose

Prior to hormone administration the blood serum had 166.1±18.7 mg/dl of glucose, which increased 34% (P<0.05), 30 minutes after the administration of glucagon. It gradually decreased, but was still 14% higher two hours after treatment (Fig. 1).

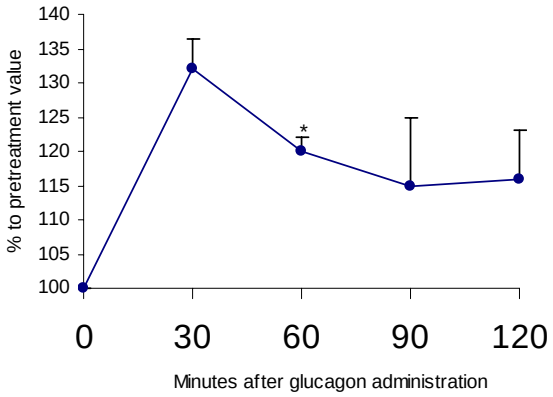

Fig.1. Plasma glucose variations following glucagon administration in induced hypoinsulinemic goats (5ug/kg body weight) *P < 0.05.

Plasma free amino acids

The plasma showed initial concentrations of FAA as 51.90±0.8 mg/dL, which showed 16% increase (P<0.05) 30 minutes and 21% 90 minutes after the treatment. This increase persisted in a narrow range for two hours following hormone infusion (Fig. 2).

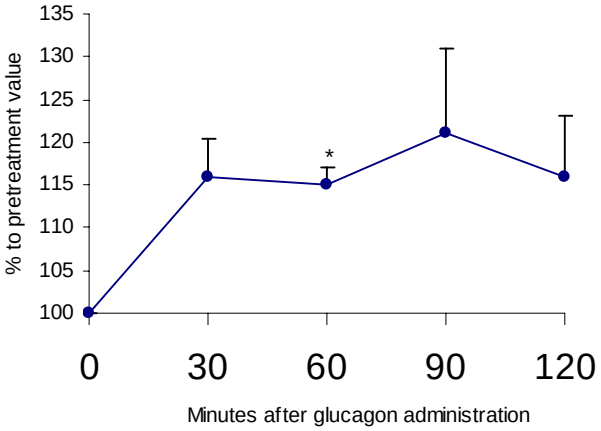

Fig.2. Plasma free amino acids variations following glucagon administration in induced hypoinsulinemic goats (5ug/kg body weight) * P < 0.05.

Plasma free fatty acids

Before glucagon infusion the concentration of plasma total FFAs was 341.1±40.1 μm/L, which were reduced 12%, one hour after the treatment (Fig. 3).

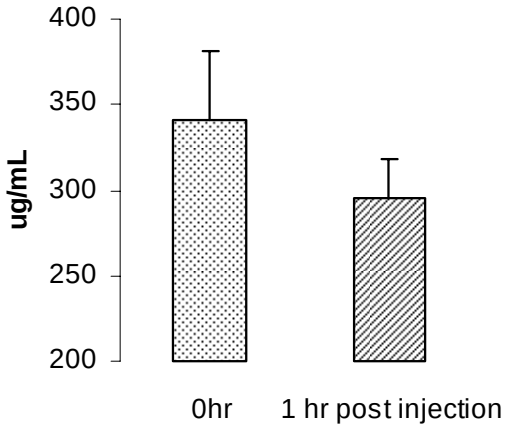

Fig. 3. Plasma total free fatty acids before and after glucagon administration with a dose of 5ug/kg body weight.

From amongst LCFAs only lauric acid (0.180 μg/mL) and palmitic acid (0.307 μg/mL) were detected before hormonal treatment. Glucagon administration caused remarkable elevation (779%) of palmitic acid. Besides that the fractions of myristic (0.35 μg/mL), stearic (4.76 μg/mL), oleic (0.69 μg/mL) and linoleic acid (1.643 μg/mL) were detected which were not detected in the sample obtained before glucagon treatment (Fig. 4).

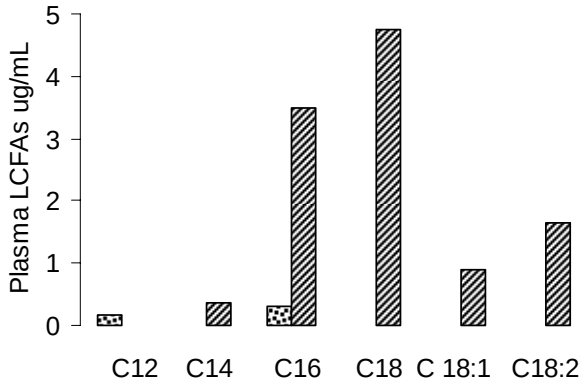

Fig. 4. Plasma long chain free fatty acids before and after glucagon administration with a dose of 5ug/kg body weight.

From amongst VFAs formic (8.615 μg/mL), acetic (213.75 μg/mL), propionic (0.1177 μg/mL), iso-caproic (0.0439 μg/mL), n-caproic (0.1757 μg/mL) and heptanoic acid (1.072 μg/mL) were detected in the sample of hypoinsulinemic goats before glucagon administration. Formic, propionic and iso-caproic acid increased remarkably 169%, 220% and 993%, respectively and n-caproic and heptanoic acid decreased considerably 80% and 23%, respectively. The fractions of iso-butyric (0.3033 μg/mL), iso-valeric (0.0296 μg/mL) and n-valeric acid (0.7262 μg/mL) also appeared which were not found in pretreatment sample (Fig. 5).

DISCUSSION

In fed hypoinsulinemic goats glucagon administration (5μg/kg body weight) elevated blood glucose 34% compared to pretreatment phase. It is an enormous response in already diabetic hyperglycemia with depleted liver glycogen. It has been well established that glucagon stimulated glycogenolysis is transient, reaching a peak within 5-30 minutes and returning to base line after 45-60 minutes (Felig *et al.,* 1976: Chiasson and Cherrington, 1983). Moreover, Chiasson *et al.* (1976) have shown that glycogenolysis was about five times more sensitive to suppression by insulin than gluconeogenesis. It also accompanied hyperaminoacedemia, which may be the main source of hyperglycemia. It has been reported by Boden *et al.* (1990) that hepatic glucose production rose only when hyperglucagonemia and hyperaminoacidemia were present together. She *et al*. (1999) have also indicated in intact state that glucagon infusions caused liver glycogenolysis initially and probably enhanced gluconeogenesis.

The glucagon is counter-regulatory to insulin in most of its biological actions is well reflected in the present study. Glucagon along with insulin in circulation displays hypoaminoacidemia as is evident by many findings. Glucagon plays an important role in the disposition of amino acids by increasing their inward transport, their degradation and their conversion into glucose (Boden *et al.,* 1990). Hyperglucagonemia enhance the catabolism of amino acids as glucagon infusion stimulate hepatic gluconeogenesis and reduce plasma amino

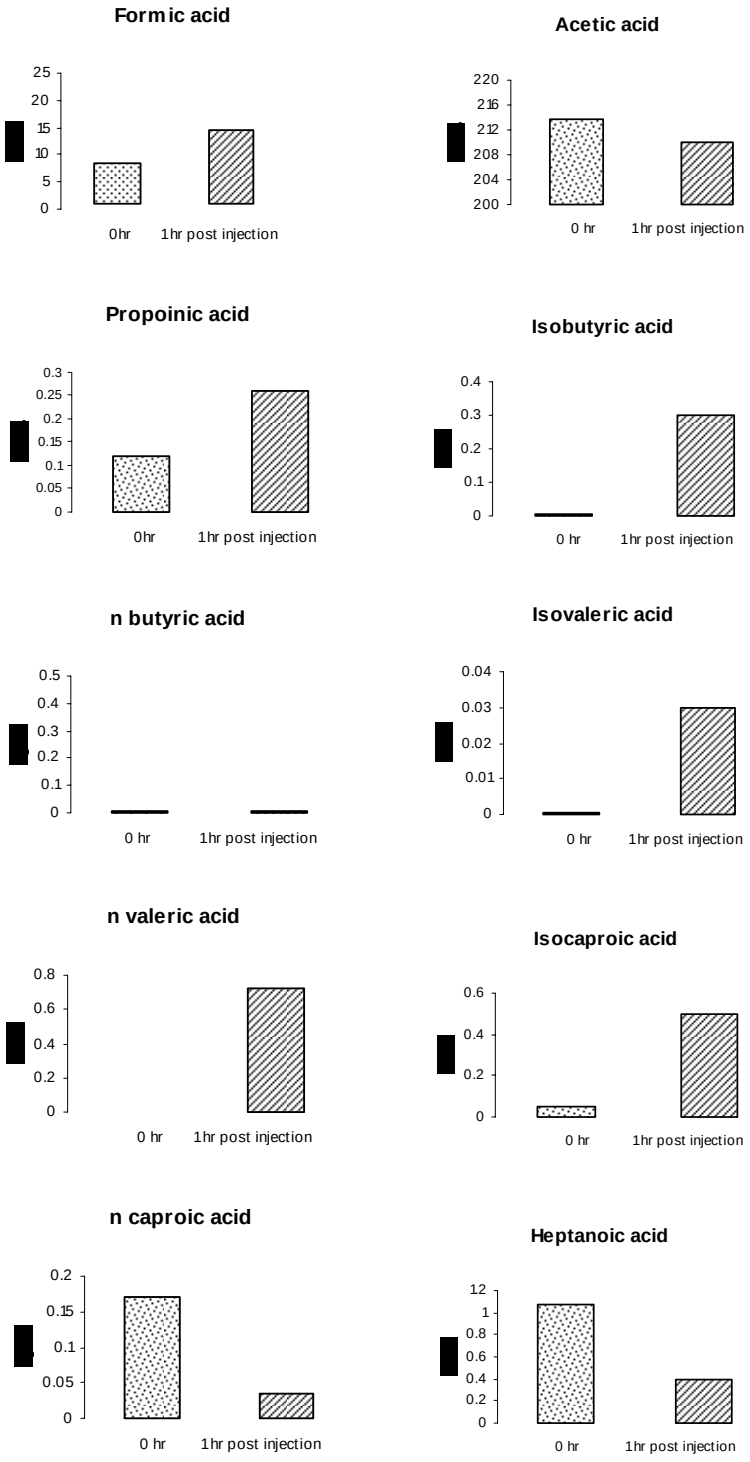

Fig.5. Circulatory levels of volatile fatty acids of low molecular weight before and after glucagon administration with a dose of 5ug/kg body weight.

acids concentration (Boden *et al.,* 1983; Gruppuso *et al.,* 1983), as well as liver and muscle FAAs (Cheema *et al.,* 1993). The results explain the observable fact that the absence of insulin in hypoinsulinemic goats caused profound hyperaminoacedemia following glucagon administration.

Plasma FFAs are decreased not significantly in hypoinsulinemic goat. Liljenquist *et al.* (1974) reported that glucagon infusion causes a marked decline in circulating FFA levels rather than an increase in normal subjects. The lipolytic action of glucagon, however in ruminants is reported in various findings as Mushtaq *et al.* (1994) have reported a decrease (15.5%) in FFA of adipose tissue following 2.5 mg /kg body weight glucagon administration in dwarf goat. The results of Bobe *et al*. (2003c) documented that subcutaneous injections of glucagon have the potential to decrease the degree of fatty liver in older dairy cows in early lactation. She *et al*. (1999) indicated that glucagon infusions caused liver glycogenolysis initially and probably enhanced gluconeogenesis but glucagon did not appear to increase lipolysis from adipose tissue in these early lactating dairy cows.

Before glucagon administration only lauric and palmitic acid were detected in the hypoinsulinemic goats, however, after hormone administration all the studied fractions appeared in circulation. It is important to note that isobutyric, n-butyric, isovaleric and n-valeric acid did not appear at all in the control sample, however, following hormone administration except n-butyric and the rest were detected in considerable amount. On the whole increasing trend in VFAs was observed in hypoinsulinemic goats with glucagon. All short chain fatty acids except acetate, stimulated glucagon secretion *in vivo* and it suggested that plasma glucagon level may be regulated by short chain fatty acids produced in the rumen (Mineo *et al.,* 1990). The release of gulcagon is less governed by VFA in free feeding goats (De-Jong, 1982).

The present study clearly indicates that fractions of LCFAs and VFAs estimated do not represent the bulk of circulatory FFA in the goat. The FFA decreased following glucagon administration (Fig. 4), whereas several fractions in LCFAs and VFAs have increased markedly. FFA probably of longer carbon chain of 18 exhibit to constitute the noticeable share in circulatory FFA pool. There are contrasting responses of the various fractions studied prior to and following glucagon administration in the hypoinsulinemic goats. A fewer fractions of VFAs and even those in low concentration were in the circulation as only lauric and palmitic acids had been detected, however, following glucagon administration, the fraction of myristic, steric and oleic acids also appeared in circulation and palmitic and showed enormous increase. The mobilization of these fractions after glucagon administration from lipid sources seem to be required for gluconeogenesis, as it is the principal response of glucagon action. How these fractions are utilized in the process can not yet be ascertained. The role of β-oxidation of these LCFAs fractions into acetate may be considered. Such possibility, however, is not confirmed from the results of acetate in the study (Fig. 5). VFAs specifically acetate and propionate the main precursor for gluconeogenesis in the ruminants have been proposed by several studies. These fractions in larger quantities are produced in rumen and transported to the liver for gluconeogenesis.

De-Jong (1982) observed that release of glucagon is less governed by VFAs, in free feeding goats. Mineo *et al.* (1990) reported that all short chain fatty acids except acetate, stimulated glucagon secretion *in vivo* and that the plasma glucagon level may be regulated by short chain fatty acids produced in rumen. The reports of effects on the responses of VFAs following glucagon did not exist before.

In the present study, it is concluded that hyperglycemia in the goat following glucagon administration is the result of both glycogenolysis and gluconeogenesis and gluconeogenesis is contributed both from the mobilized FAA and FAAs. The increase in specific FFA particularly the VFAs fractions play a part in this mechanisms. Various circulatory fractions of VFAs not worked out earlier have now been detected in the goat. Acetate did not exhibit any difference prior and after glucagon administration. However, the fractions of formic acid, propionic acid, isobutyric, isovelaric, n-valeric and iso-caproic acids increased markedly post-glucagon state, whereas n-caproic and

heptanoic acids showed decrement. There is no doubt that glucagon induces variety of variations in the studied LCFA and VFA fractions, however, mechanisms of this variability in ruminants still needs to be investigated.

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