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INTERACTION OF CIRCULATORY GLUCOSE AND FREE FATTY ACIDS IN INDUCED HYPERINSULINEMIC STATE IN DWARF GOATS *

ABDUL MAJEED CHEEMA AND RUBINA MUSHTAQ

Department of Zoology, University of the Punjab, New Campus, Lahore, Pakistan.

Abstract.- Single and repeated 3U/kg body weight long acting protamine zinc bovine insulin was administered to investigate the hormone's action on circulatory carbohydrate and lipid targets. High dose insulin caused marked ($P < 0.001$) hypoglycemia, which persistedbeyond ten hours after the treatment. Goats remained stable for nearly six hours under acute hypoglycemia but thereafter most of them experienced insulin shock, however, dextrose (20%) infusion overcame the shock in the goats. Low glycemia is the likely cause of enhanced FFAs levels as dextrose infusion in insulin shock promptly reduced the enhanced plasma FFA. Inspite of insulin resistance and alternative energy utilization of FFA, glucose is indispensable in ruminants. Strong interactions of carbohydrate and lipid targets exist in ruminants in hormonal homeostasis.

Key words: Insulin, glucose, free fatty acids, dwarf goat.

INTRODUCTION

Insulin is the most important regulator for glucose disposal and production. It affects glucose output and regulates the steady-state glucose level by altering the sensitivity of this control system (Jenkins *et al.*, 1986). Insulin inhibits glucose production with increased metabolic rate and its disposal from body (Moxley *et al.*, 1990). Ruminants demonstrate marked insulin insensitivity and hypoglycemic irresponsiveness (Kaneko, 1980; Cheema *et al.*, 1988) unlike non ruminants where insulin brings hypoglycemia with smaller doses and hepatic glycogenolysis restores glycemia. The increment in plasma insulin causing 50% reduction in glucose production is 50-60 $\mu U\ ml^{-1}$ (Brockman, 1983; Weekes *et al.*, 1983) whereas the comparable value for human is 30 $\mu U\ ml^{-1}$ (Rizza *et al.*, 1981). In ruminants hypoglycemia induced by high doses of insulin fails to return to the fasting level in two hours, which is the demonstration of hypoglycemia non-responsiveness (Cheema *et al.*, 1988) and the oral hypoglycemic agents failed to show the same degree of effectivity in regulating glucose via enhancing insulin release from β cells (Cheema *et al.*, 1989). Excess insulin may bring coma or convulsion in dogs, young ruminants and man but not the birds or mature ruminants (Hsu and Crump, 1989). Therefore insulin action on glycemia in ruminants are yet to be understood in various aspects.

Free fatty acids (FFAs) are the next target after glucose, to be most affected by insulin. Insulin is a principal antilipolytic hormone *in vivo* (Skarda and Bartos, 1969) and *in vitro* (Cochrane and Rogers, 1990). It plays distinct role in maintenance of lipogenic activity (Etherton and Evock, 1986) by increasing the rate of fatty acid synthesis in adipose tissue (de-la-Hoz and Vernon, 1993). Insulin regulates FFA inhibition of lipolysis while maintaining a constant rate of primary FFA reesterification (Campbell *et al.*, 1992). In ruminants particularly in sheep there are reports of insulin

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effects on lipid metabolism showing its antilipolytic effects (Cochrane and Rogers, 1990) and of a little effect of the hormone on fatty acid synthesis (Broad *et al.*, 1983).

It is generally agreed that glucose and lipid in metabolism are strongly correlated and this aspect is little understood in ruminants. The present study is carried out to investigate insulin action in ruminants, employing high dose/s, on glycemic and free fatty acid targets and to add information on the interaction of carbohydrate and lipid metabolism in hormonal homeostasis of ruminants based on dwarf goat model.

MATERIALS AND METHODS

Goat facility at Bio-Saline Research Sub-Station (BSRS) of Nuclear Institute for Agriculture and Biology (NIAB), Faisalabad of Pakistan Atomic Energy Commission (PAEC) was used for the study. Adult billy goats of about 2 years of old with a mean body weight of 23-29kg were used. Goats kept at a farm were shifted to the animal house and acclimatized to a feeding regime. The goats were provided with chopped green fodder from 8 am to 5 pm and dry ration for a rest of the period of the day to feed *ad libitum*. Dry ration contained composition: soya bean meal 25%, wheat bran 30%, rice polishing 27.5%, molasses 15%, urea 1%, salt 0.5% and dicalcium phosphate 1%.

Two experiments were designed for *in vivo* studies using protamine zinc bovine insulin preparation in vials (Eli Lilly Italia S.p.A. Sosto Fiorentino Firenze; Italy) in concentration of 40 U/ml for injections. In single high dose experiment goats received only one injection (intravenous) of 3.0U/kg insulin. In repeated high dose experiment, goats received an injection (3.0U/kg) again every 24 hour for three days, however, on fourth day they received glucose load (0.25g/kg body weight)) soon after insulin injection. Infusions and blood sample protocol is presented in Table 1. Serum and plasma sample obtained were stored at -20^{0}C till used for analysis.

Table 1. Bovine insulin administrations and their sampling schedule

Experiment	Hours in relation to administrations from 7:00am
Single dose (i.v.) 3.0 U/kg	-15, -1, 0↓, 0.25, 0.5, 1, 2, 4, 7S, 7.2R, 9.75S, 10R, 24
Repeated dose 3.0 U/kg (i.v.)	-15, -1, 0↓, 0.5, 6, 24↓, 24.5, 30S, 31R, 48↓, 48.5, 52S↑, 52.5R, 56S↑, 56.5R, 72↓↑, 72.25, 72.5, 73, 75, 76↑, 79, 96, 102

Insulin injection: ↓, Shock: S, Recovery: R, & Combined administration of insulin and glucose: ↓↑

Blood glucose was estimated by oxidase method (Barham and Trinder, 1972) and commercial kits (Randox Laboratories Ltd., Ardmore, U.K) were used. The method of Falholt *et al.* (1973) using copper soap formation was used for total plasma free fatty acids (FFAs). The significance of differences among the different experimental steps following administrations etc. were analyzed by one way analysis of variance (ANOVA). When F -test was significant ($P < 0.05$), contrast of the steps were tested with least significance difference [LSD] (Sokal and Rohlf, 1981).

RESULTS

FEED CONSUMPTION

Significantly reduced feed consumption during high dose insulin treatment is most likely due to the reason that goats remained uncomfortable under insulin influence. There is indication, however, of insulin role in appetite as consumption increased in days following a single dose experiment and reduction of consumption was marked when insulin was administered along dextrose.

Table 2. Total feed consumption per goat per day (dry weight in g)

Experimental state	Day 1	Day 2	Day 3	Day 4
Control phase	1102 ± 75,	789 ± 44,	1148 ± 19,	1007 ± 36
Single dose treatment phase	627 ± 17			
Post-treatment phase	1188 ± 80,	1204 ± 81,	1110 ± 103	
Repeated doses treatment phase	1004 ± 17,	707 ± 142,	852 ± 149	
Insulin + Dextrose treatment phase	418 ± 161			

GLUCOSE

Single dose: Prior to hormone administration the average concentration of plasma glucose was 60.70 ± 1.2 mg/dl. Maximum reduction of 65% ($P < 0.001$) in glycemia had occurred half an hour post-treatment, it persisted in a narrow range of fluctuations upto seven hours post-treatment, when two of the goats suffered shock, with glycemic levels 15 & 18 mg/dl. Twenty minutes after intravenous glucose infusion to these goats,

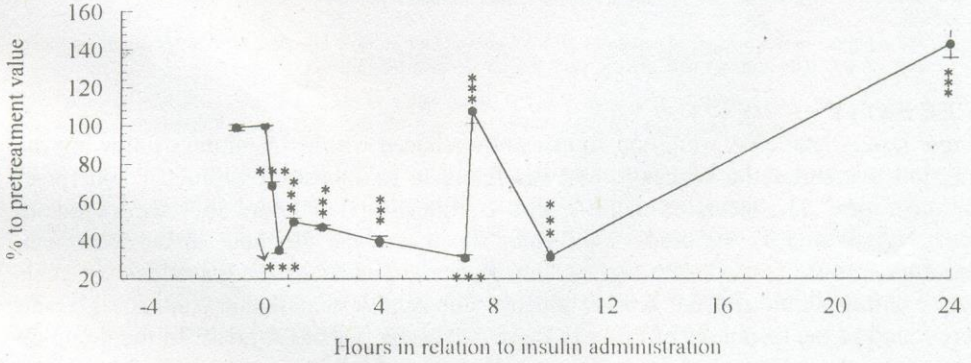

Fig. 1. Plasma glucose following single dose 3U/kg B.W. insulin. ↓ injection, *** significant level at $P < 0.001$.

glycemic level was in the range of pretreatment level. Ten hours after again hypoglycemic state returned with another shock to the same goats. Another glucose infusion kept the goats stabilized up to next morning. Twenty-four hours after glycemic

level was significantly ($P < 0.001$) greater than pre-treatment control values a day earlier (Fig. 1).

Repeated dose: Just before hormonal administration the average glycemic level was 54.05 ± 1.4 mg/dl in male dwarf goats. Marked hypoglycemia of 62% ($P < 0.001$) half an hour after injection and its persistence ($P < 0.001$) upto six hour resembled its short term pattern. All the goats remained stable during first day. With the advancing days, six hours post treatment the level was comparatively lower than the earlier day respective value, and three goats suffered insulin shock with plasma glucose 27, 19 & 23 mg/dl, thus it required glucose therapy. On the third day, one goat received fatal shock. The shock was so severe and unrecoverable that even after emergency treatments of glucose, goat died 1.75 hours after insulin injection with glycemic level 9 mg/dl. The rest of the goats received shock six hours after insulin injection from which they recovered after glucose therapy. On fourth day three hours post-combined treatment of insulin and glucose samples showed presence of severe hypoglycemia ($P < 0.001$) with the reduction of 78%; however, all the goats remained stable (Fig. 2).

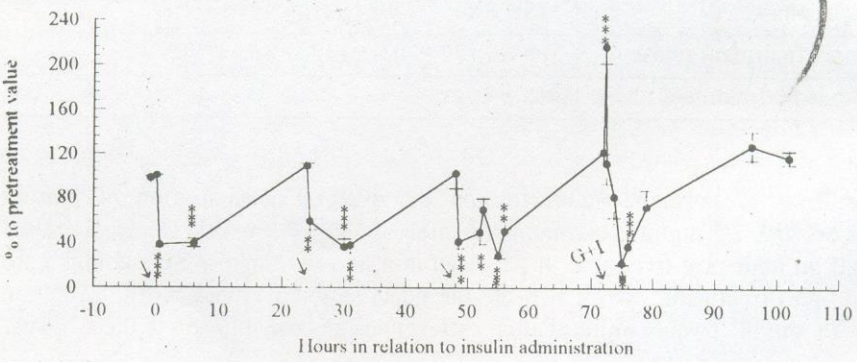

Fig. 2. Plasma glucose following repeated doses 3U/kg body weight insulin. ↓ injection, *, ** & *** significant level at $P < 0.05$, 0.01 & 0.001 respectively, G+I, insulin and dextrose infusion.

FREE FATTY ACIDS

Single dose: Total FFA were non significantly reduced within 15 minutes following the injection, thereafter the concentration was found to be increased within an hour post-administration. The elevation in FFA level continued upto 7 hours after the treatment. Goats No. 74 and 75 suffered with insulin shock at about 7th hour of the treatment. These goats were treated with intravenous glucose infusion; while the other two goats that remained stable did not receive glucose infusion. A remarkable contrasting result were found in the responses of FFAs in these two categories of the goats. In the group on glucose therapy, elevated plasma FFAs level were markedly lowered within 20 minutes after dextrose infusion. In other group which did not receive dextrose, FFA levels remained elevated. About 3 hours after the shock and glucose therapy, the lowered FFA level again increased in goats No. 74 and 75. In all the goats 24 hours after insulin treatment FFA level was found elevated than pre-treatment control values (Fig. 3).

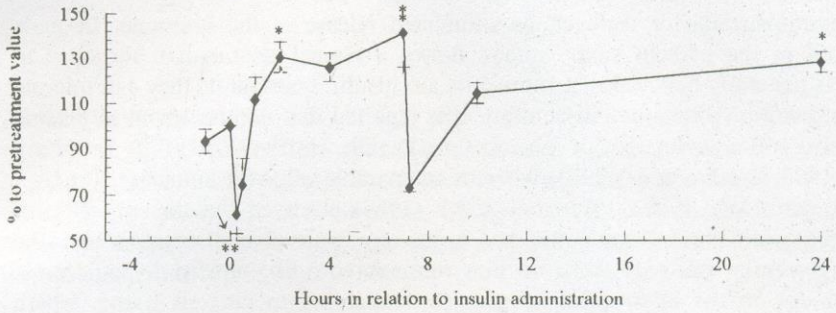

Fig. 3. Plasma free fatty acids following single dose 3U/kg body weight insulin. ↓ injection, * & ** significant level at P < 0.05, & 0.01 respectively.

Repeated Dose: The pattern in the long term treatment with high doses further confirmed the FFAs responses as found in short term high dose experiment. Within half an hour FFAs were markedly lowered (P 0.001), which later were found elevated even compare to an average control value at 6-7 hours post-treatment. The same pattern was found on second day. All the goats except 86 had suffered shock, so were given dextrose

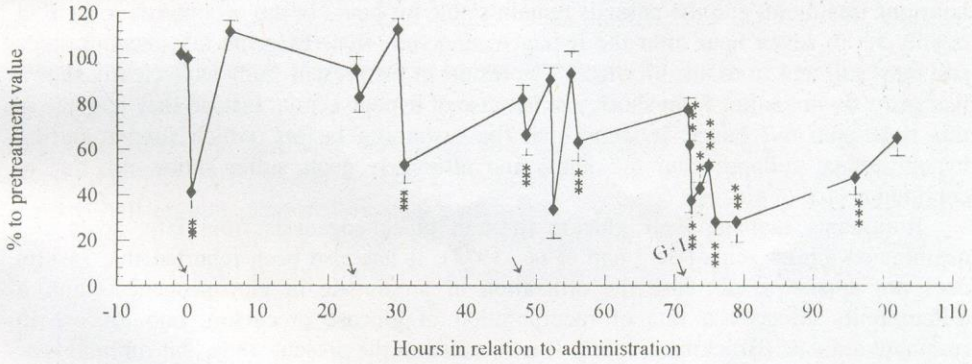

Fig. 4. Plasma free fatty acids following repeated doses 3U/kg body weight insulin. ↓ injection. *, ** & *** significant level at P < 0.05, 0.01 & 0.001 respectively, G+I, insulin and glucose infusion.

infusion. In these three goats FFAs were lowered soon after glucose administration. In goat 86 FFAs level did not decrease as it did not receive glucose therapy.

The combined administration of insulin and glucose on 4th day, confirmed the results of the earlier days that in the presence of glucose FFAs remain lower in concentration following insulin administration even upto 24 hours. Pretreatment level was restored within 30 hours after the injection (Fig. 4).

DISCUSSION

The major function of insulin is to facilitate the transport or flux of glucose across the plasma membrane of cells of most tissues, thus hypoglycemia ensues following

exogenous administration or endogenous stimulated release of the hormone. In male goats, as used in the present study, it has shown a typical mammalian response to glycemia. It is generally believed that ruminants are insulin resistant as they can tolerate high doses of insulin without any discomfort. It is reported that the increment in plasma insulin causing 50% reduction in glucose production is 50-60 μU^{ml-1} in sheep (Brockman, 1983; Weekes *et al.*, 1983) whereas comparable value for human is 30 μU^{ml-1} in man (Rizza *et al.*, 1981). Prior and Smith (1983) observed that the injection of insulin (6U/kg) into normal cattle did not bring any noticeable discomfort and the decrease in glycemia was only 22%. In non ruminant insulin induced hypoglycemic shock results due to the interruption of glucose availability to nervous tissue, which otherwise cannot store energy for emergency conditions. In ruminants, on the other hand, a fewer availability of glucose is conventionally compensated by fatty acid fractions (Preston and Leng, 1987). Therefore, ruminants have adapted to manage with very low levels of glycemia.

The long acting zinc protamine insulin, compared to regular, persists for longer duration in the circulation and can maintain hypoglycemia for several hours. In the goat, used as a model for ruminants, present study has shown that high doses of long acting insulin (3U/kg body weight) induce marked hypoglycemia within an hour after the hormone treatment, and the animals remain stable for hours in the hypoglycemic state. It is only six to seven hour after the hypoglycemic state that the goats felt uncomfortable and most suffered from insulin shock. The results of the present study have clearly shown that goats do not suffer from shock just because of hypoglycemia, instead they manage in this state upto 6-7 hours. It seems that the sustaining factors, which support during hypoglycemia, collapse after 6-7 hours and ultimately goats suffer shock also due to continuous lack of glucose.

Ruminants manage their glucose through gluconeogenesis from fatty acids and deaminated amino acids (Bergman *et al.*, 1974). It has also been reported that insulin does not appear to decrease the utilization of propionate in gluconeogenesis and it differentially affects the rate of incorporation of glucose precursors into glucose in ruminant animals (Brockman, 1990). It is revealed in the present study that ruminants or at least the dwarf goat can manage in consistent low glycemic level while using lipid constituents already present in the cells. It is also observed that the prolonged hypoglycemic condition, due to high insulin level, consequently affects adversely and inhibits the further transport of fatty acids into the cells. As the cellular energy stores are completely depleted the prolongation of the starvation of the cells for energy constituents eventually brings insulin shock even in the goat. It is clearly demonstrated in the results of present study that with the prolongation of hypoglycemia in high dose insulin treatments, plasma free fatty acids levels increased because of their inability to be transported into the cells. The reason for the lack of transport of FFA into the cells is clearly due to a low circulatory glucose, as following exogenous glucose therapy, as the level of glycemia rises, circulatory free fatty acids are declined due to their transportation into the cells. In the present study glycemic level did not reach to zero level, thus a question arises of the inability of the already available, although a low, glucose role in the circulation. This may have been due to elevated FFA as Ferrannini *et al.* (1983) and

Gomez *et al.* (1972) have observed that increased plasma FFA decrease insulin- mediated glucose uptake, glucose oxidation and glucose storage. Fetal plasma FFA increased significantly during hypoglycemia (Harwell, 1990) and also plasma FFA and urea rose in response to fasting in rumiants (Robinson *et al.*, 1992; Ward *et al.*, 1992). The results of the present study have clearly demonstrated that free fatty acids transport is adequately bound with sufficient level of circulatory glucose. There have been investigations to understand the kinetics of nutrients transport across the cell membrane. Hansen *et al.* (1992) have shown that insulin responsiveness to glucose is enhanced by simultaneous insulin exposure. Thus the transport of circulatory nutrients into the cell is just not between a single nutrient and its transporting hormone but more complex in the facilitation and inhibition rendered to one another in metabolites in relation to the hormone. It has been observed that insulin infusion into sheep portal circulation depressed glucose entry immediately (West and Passey, 1967). Hypoglycemia secondary to insulin infusion, imposes a significant stress on the organism and triggers the release of various counterregulatory hormones that tend to affect the action of insulin on glucose metabolism (Frizzell *et al.*, 1988; Gerich *et al.*, 1979). It is associated with enhanced hepatic glucose production, lipolysis, and ketogenesis (Frizzell *et al.*, 1988). Insulin is understood to increase the rate of fatty synthesis in adipose tissue (de-la-Hoz and Vernon, 1993).

It is evident, from the study, that inspite of insulin resistance and even in alternate source of energy of FFA, in intense hypoglycemia, due to prolonged insulin action, glucose is still indispensable for, at least, free fatty acid transport into the tissues of ruminants. Also an intense carbohydrate and lipid metabolic interaction is espied in these animals.

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