

## The relative roles of calcium, phosphorus, and parathyroid hormone in glucose- and tolbutamide-mediated insulin release.

H R Harter, ... , E Slatopolsky, S Klahr

*J Clin Invest.* 1976;58(2):359-367. <https://doi.org/10.1172/JCI108480>.

### Research Article

The relative contributions of  $\text{Ca}^{++}$ , phosphorus, and parathyroid hormone (PTH) on insulin secretion were evaluated in three groups of dogs. Dogs were studied with glucose infusions (group I) or standard intravenous glucose tolerance tests (IVGTT) (group II) before and after the development of diet-induced hypophosphatemia. Mean serum phosphorus levels for both groups fell from 4.1 to 1.1 mg/100 ml. Animals in group I demonstrated a fall in glucose disappearance rates ( $K_g$ ) from  $5.3 \pm 0.6\%$  min to  $3.5 \pm 0.5\%$  after induction of hypophosphatemia ( $P$  less than 0.001). Mean insulin response was significantly greater in the hypophosphatemic animals than in controls in this group. In group II animals, mean insulin areas obtained during the IVGTT increased from  $1,426 \pm 223$  to  $2,561 \pm 141$   $\mu\text{U}/\text{ml}/60$  min after induction of hypophosphatemia, and were unaffected by  $\text{Ca}^{++}$  or PTH administration.  $\text{Ca}^{++}$  administration, but not hypophosphatemia or PTH infusion, increased significantly the mean insulin response to tolbutamide. Secondary hyperparathyroidism was induced by dietary manipulation in four dogs (group III). Mean PTH values increased from  $71.4 \pm 2.1$  to  $3,012 \pm 372$  pg/ml ( $P$  less than 0.001). Mean insulin response to an IVGTT was similar to group III animals, but increased from  $1,352 \pm 128$  to  $1,894 \pm 360$   $\mu\text{U}/\text{ml}/60$  min after the excessive dietary phosphorus was reduced for 3 mo, and plasma phosphorus fell from  $3.2 \pm 0.1$  to  $2.8 \pm 0.3$  mg/100 ml. PTH values decreased to  $647 \pm 53$  pg/ml. The insulin response [...]

Find the latest version:

<https://jci.me/108480/pdf>



# The Relative Roles of Calcium, Phosphorus, and Parathyroid Hormone in Glucose- and Tolbutamide-Mediated Insulin Release

H. R. HARTER, J. V. SANTIAGO, W. E. RUTHERFORD, E. SLATOPOLSKY, and S. KLAHR

*From the Renal Division and Division of Endocrinology, Departments of Medicine and Pediatrics, Washington University School of Medicine, St. Louis, Missouri 63110*

**ABSTRACT** The relative contributions of  $\text{Ca}^{++}$ , phosphorus, and parathyroid hormone (PTH) on insulin secretion were evaluated in three groups of dogs. Dogs were studied with glucose infusions (group I) or standard intravenous glucose tolerance tests (IVGTT) (group II) before and after the development of diet-induced hypophosphatemia. Mean serum phosphorus levels for both groups fell from 4.1 to 1.1 mg/100 ml. Animals in group I demonstrated a fall in glucose disappearance rates ( $K_g$ ) from  $5.3 \pm 0.6\%$ /min to  $3.5 \pm 0.5\%$  after induction of hypophosphatemia ( $P < 0.001$ ). Mean insulin response was significantly greater in the hypophosphatemic animals than in controls in this group. In group II animals, mean insulin areas obtained during the IVGTT increased from  $1,426 \pm 223$  to  $2,561 \pm 141$   $\mu\text{U}/\text{ml}/60$  min after induction of hypophosphatemia, and were unaffected by  $\text{Ca}^{++}$  or PTH administration.  $\text{Ca}^{++}$  administration, but not hypophosphatemia or PTH infusion, increased significantly the mean insulin response to tolbutamide.

Secondary hyperparathyroidism was induced by dietary manipulation in four dogs (group III). Mean PTH values increased from  $71.4 \pm 2.1$  to  $3,012 \pm 372$  pg/ml ( $P < 0.001$ ). Mean insulin response to an IVGTT was similar to group III animals, but increased from  $1,352 \pm 128$  to  $1,894 \pm 360$   $\mu\text{U}/\text{ml}/60$  min after the excessive dietary phosphorus was reduced for 3 mo, and plasma phosphorus fell from  $3.2 \pm 0.1$  to  $2.8 \pm 0.3$  mg/100 ml. PTH values decreased to  $647 \pm 53$  pg/ml. The insulin

response to tolbutamide was comparable to that in group II animals, but increased significantly after calcium administration. Immunoreactive insulin disappearance rates were unaffected by hypophosphatemia or diet-induced secondary hyperparathyroidism.

These data demonstrate that hypophosphatemia is associated with an augmented glucose-stimulated insulin release, without any effect on tolbutamide-stimulated insulin release. Hypercalcemia produces an augmented tolbutamide-stimulated insulin release with no apparent effect on glucose-stimulated insulin release. Finally, PTH does not appear to be an insulin antagonist and has no apparent effect on either glucose- or tolbutamide-stimulated insulin release in animals with dietary-induced secondary hyperparathyroidism.

## INTRODUCTION

Although previous studies have demonstrated both carbohydrate intolerance and hyperinsulinemia in primary hyperparathyroidism (1, 2), the mechanisms underlying these abnormalities remain unclear. Hyperparathyroidism is associated with a complex variety of metabolic disturbances that include elevated levels of parathyroid hormone (PTH),<sup>1</sup> variable hypercalcemia and hypophosphatemia, and mild hyperchloremic acidosis (3). The relative effects of each of these abnormalities on carbohydrate intolerance and insulin secretion have not been systematically studied. It has been shown that acute administration of PTH to human volunteers has no apparent effect on glucose-mediated insulin release (1). Furthermore, while chronic PTH administration

This paper was presented in part at the Midwest Section, American Federation of Clinical Research, Chicago, Ill., November 1975.

Received for publication 24 November 1975 and in revised form 25 March 1976.

<sup>1</sup> Abbreviations used in this paper: IVGTT, intravenous glucose tolerance test;  $K_g$ , glucose disappearance rate; PTH, parathyroid hormone.

caused increased glucose and tolbutamide-mediated insulin secretion, PTH addition to isolated rat pancreatic islets incubated *in vitro* had no effect on insulin release (1). Acute hypercalcemia did not affect glucose-stimulated insulin secretion, but it increased the release of insulin in response to tolbutamide (1). It has been postulated, therefore, that chronic hypercalcemia may represent the primary mechanism responsible for the hyperinsulinemia and, perhaps, the carbohydrate intolerance seen in primary hyperparathyroidism (1, 2), and in other abnormalities associated with abnormal PTH secretion and plasma calcium levels (2, 4).

Preliminary studies from our laboratory demonstrated that hypophosphatemia was associated with an augmented insulin response to a given glucose stimulus, independent of either hypercalcemia or elevated PTH levels (5). Furthermore, the glucose disappearance rate ( $K_g$ ) after cessation of a glucose infusion was decreased in the hypophosphatemic animals (6). The present experiments, therefore, were designed to systematically study the effect of independent changes in serum calcium, PTH, and phosphorus levels on the glucose tolerance and insulin secretory response in dogs.

## METHODS

A total of 133 studies were performed on 20 normal female mongrel dogs. The animals were divided into three groups.

*Group I. Glucose infusion studies in normal and hypophosphatemic dogs.* To evaluate prolonged insulin response, eight dogs weighing 12–23 kg were studied before and after the induction of hypophosphatemia. Three dogs were studied initially and after 2 mo on the same diet with supplemental phosphate to prevent the development of hypophosphatemia. Glucose infusions were used, since it has recently been demonstrated that dog insulin response to this challenge is associated with continuing increases in plasma insulin concentrations without an obvious peak response (7). All animals were fed a low-phosphorus synthetic diet, to which 1,200 mg of phosphorus as neutral phosphate were added (ICN Nutritional Biochemicals, Div. International Chemical & Nuclear Corp., Cleveland, Ohio, diet 1120). The diet contained 20% protein as blood fibrin, 70% carbohydrate as sucrose, and 10% fat as butterfat. Essential vitamins and a salt mixture containing appropriate amounts of trace minerals and 5 g sodium chloride were added. Each animal was tube fed 400 g of the diet (1,600 cal) per day, which conforms to the recommended (Ralston Purina Experimental Lab, Ralston Purina Co., St. Louis, Mo.) caloric intake for dogs (40–50 cal/lb body wt). After at least 2 wk on this diet, the animals were fasted overnight and studied while awake, resting quietly in a sling. On the morning of the study, catheters were placed in the femoral artery for blood sampling and in the bladder for urine collections. After placement of the catheters, 1 h of equilibration was allowed before the animals were infused with dextrose-containing solutions at 5 ml/min via a hind leg vein. Each concentration of dextrose in water (2.5, 8, 11, 15, 20, and 30%) was infused for 30 min. Arterial samples were obtained by continuous drip in 10-min intervals for all determinations through the course of the infusions. A blood glucose level of at least 450–500 mg/100

ml was reached in each animal at the termination of the infusion. At this time, the infusions were discontinued and arterial samples were obtained every 10 min until the blood sugar had returned to normal, for calculation of plasma  $K_g$ , by the formula  $K_g = 0.693/t\frac{1}{2}$ . Only glucose values below 300 mg/100 ml were used for these calculations. The arterial samples were also analyzed for insulin, total and ionized calcium, and phosphorus, as well as for PTH values.

In three of these animals, the glucose infusions were repeated after the dogs had been maintained 60–70 days on the low-phosphate diet with supplemental phosphate. No hypophosphatemia was present in these animals. In eight dogs, after base-line studies, the animals were fed the same low-phosphate diet for 90–120 days, but the supplemental phosphorus was omitted and aluminum carbonate gel (90 ml/day) was added. The animals were restudied at serum phosphorus levels of 1–1.5 mg/100 ml, and when the serum ionized calcium levels, after initial increases, had stabilized in the normal range. These animals gained weight, from  $17.0 \pm 0.9$  to  $17.9 \pm 0.7$  kg. They appeared healthy and had no detectable changes in blood potassium, magnesium, or hematocrit values after the induction of hypophosphatemia. The dextrose infusions were repeated as outlined above. These infusions were then repeated in the hypophosphatemic animals after PTH (Lilly Experimental, Eli Lilly and Company, Indianapolis, Ind., lot 4U247) administration. The PTH was given as a 200-U bolus 1 h before the glucose infusions. Thereafter a continuous infusion of PTH was given at 2 U/min during the 1-h equilibration period and the glucose infusions. Additional arterial samples were obtained every 30 min in these animals to document the elevation of plasma PTH levels.

*Group II. Intravenous glucose, insulin, and tolbutamide tolerance tests in normal and hypophosphatemic dogs.* In another group of eight dogs, weighing 16–24 kg, additional studies were performed. These animals were fed the synthetic diet as outlined for group I and were studied after an overnight fast. Approximately 1 wk was allowed between studies. Jugular venous catheters were used for blood sampling, while a hind leg vein was used for all infusions.

Intravenous glucose tolerance tests (IVGTT) were performed after administering 500 mg/kg body wt of 50% dextrose over 30 s, and blood was collected at 0, 2, 5, 10, 20, 30, 40, 50, and 60 min to calculate the  $K_g$  and the insulin response. The IVGTT studies were repeated in these animals 1 wk later in an identical fashion except that  $\text{CaCl}_2$  was given at 2 mg/kg/h 1 h before and during the IVGTT to evaluate the effects of acute hypercalcemia on the parameters being studied, independently of the plasma PTH levels. 1 wk later, a PTH prime of 200 U, followed by a continuous infusion of 2 U/min, was given for 1 h before and during the IVGTT's in four of the animals.

Insulin tolerance tests were performed by infusing 0.1 U/kg body wt of regular insulin intravenously and collecting blood at 10-min intervals to measure glucose levels and insulin disappearance rates in these same four animals. The studies were repeated after the administration of either calcium or PTH as described above.

Tolbutamide tolerance tests were performed by administering 500 mg of tolbutamide over 30 s, after it had been determined that this dose of tolbutamide produced a 50% fall in plasma glucose in normal dogs. These studies were repeated after the administration of either calcium or PTH as described above.

After all base-line studies had been completed, hypophos-

TABLE I  
*Mean Serum Phosphorus, Total and Ionized Calcium, and PTH Values for the Control and Hypophosphatemic Animals of Groups I and II*

Condition	Phosphorus <i>mg/100 ml</i>	Ionized calcium <i>mg/100 ml</i>	Total calcium <i>mg/100 ml</i>	PTH <i>pg/ml</i>
Control ( <i>n</i> = 16)	4.1±0.1	4.7±0.1	10.4±0.1	71.4±2.1
Calcium infusion ( <i>n</i> = 8)	3.8±0.1	5.4±0.2*	10.9±0.2‡	Not performed
PTH infusion ( <i>n</i> = 4)	4.2±0.3	4.6±0.1	10.4±0.2	Not performed
Hypophosphatemic ( <i>n</i> = 16)	1.1±0.2*	4.9±0.1	10.4±0.2	Undetectable
Calcium infusion ( <i>n</i> = 4)	1.1±0.6*	5.9±0.5*	11.6±0.2*	Undetectable
PTH infusion ( <i>n</i> = 12)	0.9±0.1*	5.2±0.2‡	10.8±0.5	7,463±476*

Group I animals received the glucose infusions and group II animals received the IVGTT. *n* refers to the number of animals studied.

\* Significantly different from control at  $P < 0.001$ .

‡ Significantly different from control at  $P < 0.025$ .

phatemia was induced, as in group I animals, and all studies were then repeated.

*Group III. Secondary hyperparathyroidism studies.* Secondary hyperparathyroidism was induced in a third group of four dogs (8) to test the effect of a chronic increase of plasma PTH on glucose tolerance and insulin release. The animals were fed 400 g/day (1,600 cal) of a low-calcium diet (Nutritional Biochemical, diet 101214), containing 16 mg of calcium and 1,920 mg of phosphorus daily, to which 20 g of phosphate as neutral phosphate were added. The remaining dietary constituents were sucrose 68%, casein 24%, and fat 8%. The diet also contained 5 g of sodium chloride and essential minerals and vitamins. After approximately 5 mo on this diet, serum PTH levels had risen over 20-fold to 3,012±372 pg/ml, and all studies were performed at this time. All animals were studied as described above for group II, with base-line intravenous glucose, insulin, and tolbutamide tolerance tests. The tolbutamide tolerance tests were repeated during a simultaneous calcium infusion, as described above. After these studies had been completed, the dogs were fed the low-calcium diet without the added neutral phosphate. Approximately 3 mo later, serum phosphorus levels had fallen 20% to 2.8±0.3 mg/100 ml, while PTH levels had fallen 78% to 647±53 pg/ml. The glucose, insulin, and tolbutamide tolerance tests were repeated, as outlined above, at this time.

*Laboratory determinations.* Plasma and urinary glucose concentrations were determined with the glucose oxidase Autoanalyzer (Beckman Instruments Inc., Fullerton, Calif.). Serum phosphorus and total and ionized calcium levels were measured as previously described (9). Plasma insulin was determined by a double-antibody radioimmunoassay with human insulin as the standard and <sup>125</sup>I-porcine insulin as the tracer. Numerous preliminary assays comparing charcoal-treated dog plasma and EDTA buffer revealed duplicate displacement curves, and for this reason EDTA buffer was utilized in all assays. Guinea pig antibody preparations and standard curves were diluted in 1% bovine serum albumin. The interassay coefficient of variation was 14.1%. Plasma PTH was determined by previously described techniques (10). Statistical analyses were performed by Student's *t* analysis for paired or unpaired samples where applicable. All data presented are means±SE.

## RESULTS

*Serum phosphorus, calcium, and PTH values in dogs of groups I and II.* The serum phosphorus, ionized calcium, total calcium, and serum PTH values for the animals of groups I and II were similar and have therefore been combined in Table I. Serum phosphorus levels fell from 4.1±0.1 to 1.1±0.2 mg/100 ml during phosphorus restriction ( $P < 0.001$ ), and were not affected significantly by either calcium or PTH administration in the normal or hypophosphatemic state. Despite the significant fall in serum phosphorus, ionized calcium levels during hypophosphatemia (4.9 mg/100 ml) were not significantly different from control values (4.7 mg/100 ml). Mean ionized calcium levels were increased 0.7 and 1.0 mg/100 ml in the control and hypophosphatemic states, respectively, during calcium administration. PTH infusion increased ionized calcium levels in the hypophosphatemic state, but not in the normal state. Serum PTH values fell to undetectable levels in the hypophosphatemic state and increased markedly (7,463±476 pg/ml) after PTH administration.

*Glucose infusion studies (group I).* Fig. 1 illustrates the mean plasma insulin concentration for eight animals studied in the normal and hypophosphatemic states during continuous graded glucose infusions. During the infusions, plasma glucose concentrations increased linearly in all control and hypophosphatemic dogs, although somewhat more rapidly in the latter group. The increasing glucose values were plotted against the respective insulin response for each dog. The insulin values at glucose concentrations of 100, 200, and 300 mg/100 ml were then calculated for each dog. Fig. 1 represents the mean of all values obtained in these eight animals.

Insulin values were similar at plasma glucose con-

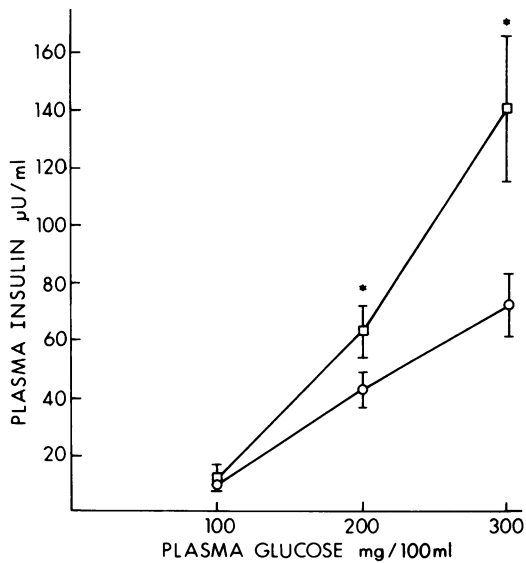


FIGURE 1 Mean insulin responses in eight normal (○—○) and hypophosphatemic (□—□) animals given a continuous graded glucose infusion. The asterisks mark values significantly different from control at  $P < 0.025$ .

centrations of 100 mg/100 ml or less, but were significantly higher ( $P < 0.025$ ) at glucose levels of 200 and 300 mg/100 ml in the hypophosphatemic dogs. Despite a significant increase in insulin response in the hypophosphatemic animals,  $K_d$  values obtained after termination of the glucose infusion from blood glucose levels below 300 mg/100 ml only were significantly lower ( $3.5 \pm 0.5$  vs.  $5.3 \pm 0.4\%$ /min) ( $P < 0.001$ ) in the hypophosphatemic dogs (Table II). Thus, hypophosphatemic dogs given a continuous graded dextrose infusion generate a greater insulin response to a given blood glucose concentration and have an impaired  $K_d$  after cessation

TABLE II

Mean Glucose Disappearance Rates ( $K_d$ ) Obtained from Normal and Hypophosphatemic Animals in Groups I and II

Condition	$K_d$	
	Group I	Group II
	$\%/min$	
Control	$5.3 \pm 0.6$ ( $n = 8$ )	$2.2 \pm 0.1$ ( $n = 8$ )
Calcium infusion	—	$2.2 \pm 0.2$ ( $n = 8$ )
PTH infusion	—	$2.3 \pm 0.1$ ( $n = 4$ )
Hypophosphatemic	$3.5 \pm 0.5^*$ ( $n = 8$ )	$2.4 \pm 0.2$ ( $n = 8$ )
Calcium infusion	—	$2.3 \pm 0.2$ ( $n = 4$ )
PTH infusion	$3.6 \pm 0.4^*$ ( $n = 6$ )	$2.1 \pm 0.2$ ( $n = 4$ )

For an explanation of groups I and II, refer to Table I.  $n$  refers to the number of animals studied.

\*Significantly different from the group I control value at  $P < 0.001$ .

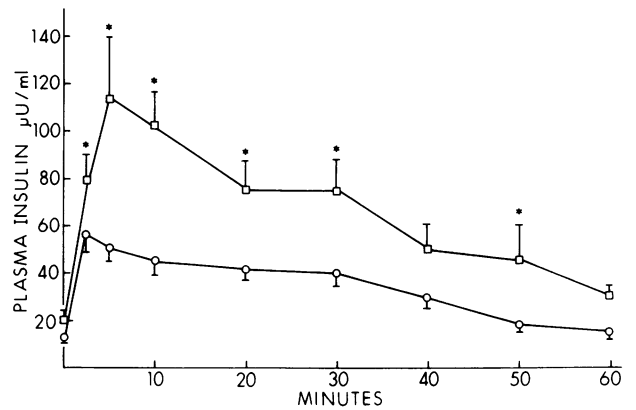


FIGURE 2 Mean plasma insulin responses in eight normal (○—○) and hypophosphatemic (□—□) animals obtained during IVGTT. \*Values significantly different from control at  $P < 0.025$ .

of the infusion. In the three animals maintained on the same diet but with supplemental phosphate, the insulin response was not significantly altered after 2 mo on this diet.

Acute PTH administration had no effect on either the insulin response (data not shown) or  $K_d$  values (Table II) in the hypophosphatemic dogs.

IVGTT (group II). The mean glucose disappearance rates obtained during IVGTT's in eight dogs studied in the normal and hypophosphatemic states were unaffected by hypophosphatemia or by acute calcium or PTH administration (Table II). This difference in glucose disappearance as compared to group I animals could be because a much larger total glucose load was given during infusions than during the glucose tolerance tests. The mean total insulin response (Fig. 2) was significantly increased in hypophosphatemic animals. Fig. 3 illustrates the mean insulin area above base-line for

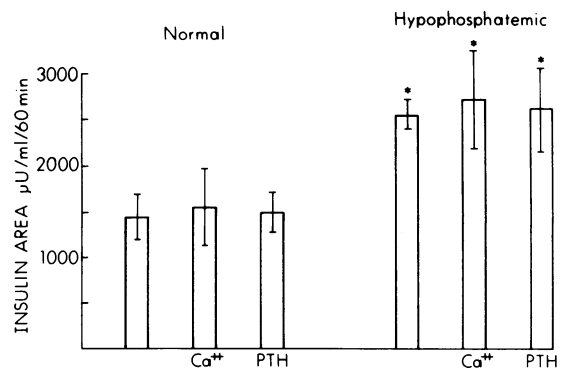


FIGURE 3 Mean insulin areas above base line obtained from eight normal and hypophosphatemic animals with and without calcium ( $Ca^{++}$ ) or PTH infusions. \*Values significantly different from the corresponding control values at  $P < 0.001$ .

normal ( $1,426 \pm 223 \mu\text{U/ml/60 min}$ ) and hypophosphatemic ( $2,561 \pm 141 \mu\text{U/ml/60 min}$ ,  $P < 0.001$ ) animals obtained during the IVGTT's. The insulin response was essentially unchanged during calcium or PTH administration in normal animals ( $1,644 \pm 428 \mu\text{U/ml/60 min}$  and  $1,505 \pm 221 \mu\text{U/ml/60 min}$ , respectively). Similarly, insulin responses in hypophosphatemic animals were unaffected by calcium ( $2,738 \pm 569 \mu\text{U/ml/60 min}$ ) or PTH ( $2,540 \pm 394 \mu\text{U/ml/60 min}$ ) administration. Insulin areas were calculated with a standard computer program and were verified by planimetry. Analysis of the combined basal insulin data from groups I and II revealed that normal animals had a basal insulin value of  $11.6 \pm 0.8 \mu\text{U/ml}$  and the hypophosphatemic animals had a basal insulin level of  $17.2 \pm 1.0 \mu\text{U/ml}$  ( $P < 0.001$  compared to control).

**Insulin tolerance test (group II).** After intravenous insulin administration, the plasma immunoreactive insulin disappearance rates were similar in control and hypophosphatemic dogs ( $t_{1/2}$  value of  $8.6 \pm 0.6$  and  $9.3 \pm 0.7$  min, respectively). These results were not statistically different. Administration of calcium or PTH to either the normal or hypophosphatemic animals did not significantly affect the disappearance rates of immunoreactive insulin. Fig. 4 illustrates the hypoglycemic response of both normal and hypophosphatemic animals to insulin administration. The hypophosphatemic animals demonstrated a comparable percent decline in plasma glucose, but a more rapid return of plasma glucose levels to normal when compared to control. Neither calcium nor PTH administration had any significant effect on the degree of hypoglycemia or the rate of recovery in normal or hypophosphatemic dogs.

**Tolbutamide tolerance test.** The effect of tolbutamide on insulin secretion was evaluated in four dogs in the control and hypophosphatemic state. Mean insulin se-

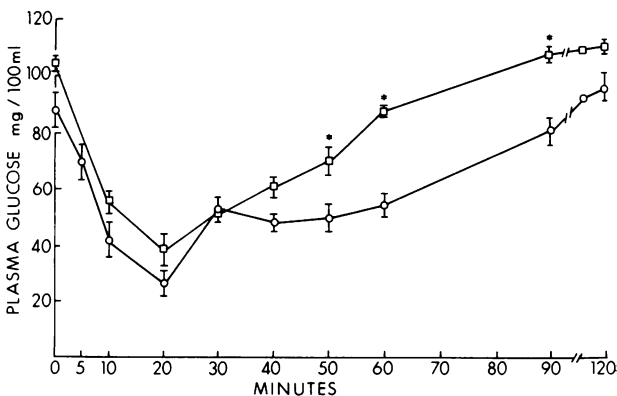


FIGURE 4 Hypoglycemic response in four normal (○—○) and hypophosphatemic (□—□) animals given intravenous insulin. \* Values significantly different from control at  $P < 0.05$ .

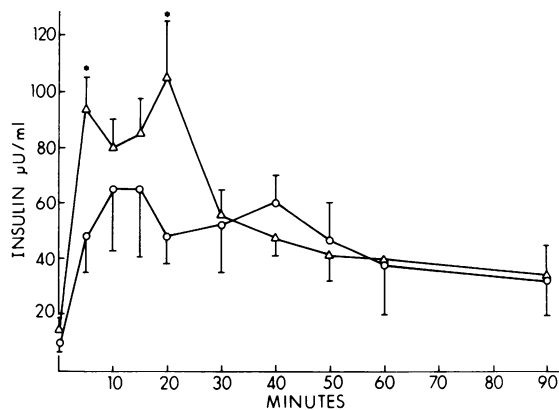


FIGURE 5 Plasma insulin responses in four normal animals given intravenous tolbutamide before (○—○) and after (△—△) calcium administration. \* Values significantly different from control at  $P < 0.05$ .

cretory responses were similar in both groups ( $1,947 \pm 122 \mu\text{U/ml/60 min}$  in controls and  $1,818 \pm 485 \mu\text{U/ml/60 min}$  in hypophosphatemic animals). The hypoglycemic response of normal and hypophosphatemic animals were not significantly different. Interestingly, calcium administration to normal animals significantly increased the early peak insulin response to tolbutamide (Fig. 5) as well as total insulin area ( $2,948 \pm 570 \mu\text{U/ml/60 min}$ ) ( $P < 0.05$ ). On the other hand, PTH administration to normal or hypophosphatemic animals had no effect on tolbutamide stimulated insulin release.

**Dietary hyperparathyroidism studies (group III).** Table III depicts the mean serum phosphorus, total and ionized calcium, and PTH levels in dogs made hyperparathyroid by dietary manipulation (group III). For comparison, the values obtained from normal animals are also included. The hyperparathyroid animals had serum phosphorus levels within the normal range for our laboratory ( $3.2 \text{ mg/100 ml}$ ), but still significantly lower than control values ( $4.1 \text{ mg/100 ml}$ ) and probably reflecting the 10-h fast in animals with very high PTH values ( $3,012 \pm 275 \text{ pg/ml}$ ) ( $P < 0.001$  compared to control). Ionized calcium remained within the normal range and  $K_d$  values ( $2.5 \pm 0.2\%/min$ ) were comparable to those of normal animals.

After elimination of the excessive dietary phosphorus for 4 mo, plasma phosphorus levels fell to  $2.8 \pm 0.3 \text{ mg/100 ml}$ , a value not significantly different from that obtained in the hyperparathyroid animals fed a high phosphorus diet. The PTH values fell 79% to  $647 \pm 53 \text{ pg/ml}$  ( $P < 0.001$ ), a value, however, still significantly greater than that of normal dogs ( $P < 0.001$ ). The  $K_d$  ( $2.47 \pm 0.2\%/min$ ) and ionized calcium levels obtained during the IVGTT remained within the normal range. Fig. 6 illustrates the mean insulin areas in four hyperparathyroid animals before and after elimination of the ex-

TABLE III  
Mean Serum Calcium, Phosphorus, and PTH Values for Animals of Groups I and II and Group III

Diet	Phosphorus mg/100 ml	Ionized calcium mg/100 ml	Total calcium mg/100 ml	PTH pg/ml
Low calcium, high phosphorus, (n = 4), group III	3.2±0.1‡	4.6±0.1	10.1±0.1	3,012±372*
Low calcium, normal phosphorus, (n = 4), group III	2.8±0.3‡	4.7±0.2	9.9±0.1	647±53*
Normal calcium, normal phosphorus, (n = 16), groups I and II	4.1±0.1	4.7±0.1	10.4±0.1	71.4±2.1

Group I animals received intravenous glucose infusions. Group II animals received IVGTT. Group III animals were hyperparathyroid and received IVGTT. *n* refers to the number of animals studied.

\* Significantly different from groups I and II at  $P < 0.001$ .

‡ Significantly different from groups I and II at  $P < 0.05$ .

cessive dietary phosphorus. Three of four animals demonstrated a significant increase of mean insulin response to the IVGTT. Mean insulin response during the IVGTT for all four hyperparathyroid animals was  $1,352 \pm 128$   $\mu\text{U}/\text{ml}/60$  min before and  $1,894 \pm 360$   $\mu\text{U}/\text{ml}/60$  min after phosphorus restriction. Thus, despite a significant reduction in plasma PTH levels associated with a fall in plasma phosphorus, total insulin response increased.

The insulin responses to tolbutamide testing in the hyperparathyroid animals were not significantly different from control ( $1,476 \pm 161$   $\mu\text{U}/\text{ml}/60$  min). Interestingly, however, during calcium administration this response increased significantly to  $2,318 \pm 342$   $\mu\text{U}/\text{ml}/60$

min. The hypoglycemic responses to both tolbutamide and insulin administration were not significantly different from control, and were unaffected by calcium administration. The immunoreactive insulin clearances ( $t_{1/2} = 8.4 \pm 0.1$  min) were unaffected by hyperparathyroidism.

## DISCUSSION

Primary hyperparathyroidism may be associated with many metabolic abnormalities, including hypercalcemia, hypophosphatemia, hyperchloremic acidosis, and elevated levels of PTH (3). Recent studies have demonstrated increased insulin secretion in response to tolbutamide or glucose challenge in primary hyperparathyroidism (1), and it has been suggested that the augmented insulin responsiveness may be related to elevated plasma calcium levels. Investigators have also postulated a role for calcium as a modulator of glucose and tolbutamide-stimulated insulin release from pancreatic islets in vitro (11–17). Although Kim et al. (1) demonstrated that PTH had no effect on insulin release from isolated pancreatic islets, a possible insulin antagonistic effect was not excluded in patients with primary hyperparathyroidism. Also, in patients with uremia and secondary hyperparathyroidism, an insulin antagonistic role of PTH was suggested (18), but was not confirmed by other investigators (4). The possible relationship of phosphorus to the increased insulin release of primary hyperparathyroidism was not discussed by previous investigators. The present studies demonstrate that in the dog (a) hypophosphatemia is associated with augmented glucose-stimulated insulin release and mild glucose intolerance, independent of changes in plasma ionized calcium levels; (b) PTH has no apparent direct effect on glucose- or tolbutamide-stimulated insulin release; (c) PTH does not appear to be an insulin antagonist in this animal model; and (d)

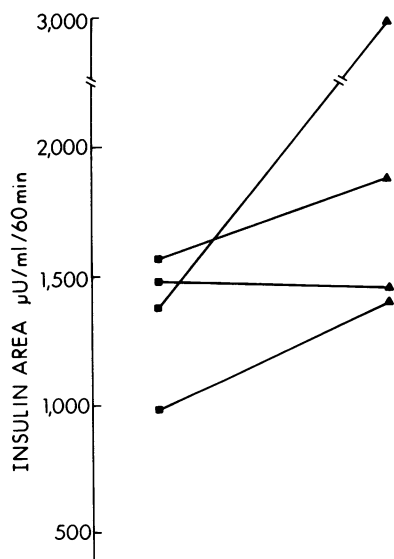


FIGURE 6 Insulin area above base line for four hyperparathyroid animals obtained during IVGTT before (■—■) and after (▲—▲) restriction of dietary phosphorus.

calcium augments tolbutamide-stimulated insulin release in normal and hyperparathyroid animals, but has no apparent effect on glucose-stimulated insulin release within the plasma concentrations studied.

Hypophosphatemia in our animals was associated with undetectable plasma PTH and normal total and ionized calcium levels (Table I), findings previously described by other investigators (19). An augmented insulin response to a glucose challenge was noted in the hypophosphatemic state (Fig. 1-3), which was also associated with a depressed  $K_d$  after cessation of the glucose infusions (Table II). Furthermore, the hypophosphatemic animals had a significantly higher basal insulin level than the normal animals. This demonstrates a mild impairment of glucose utilization in the hypophosphatemic animal, and suggests some degree of insulin resistance, since immunoreactive insulin clearance rates were normal, although this was not confirmed by the insulin tolerance tests. This may, however, reflect the mild nature of the resistance and the excessive dose of insulin used.

Acute calcium administration induced no changes in glucose disappearance rates during the IVGTT (Table II) or in total insulin response to a glucose challenge (Fig. 3) in either normal or hypophosphatemic animals. Induction of hypercalcemia, however, significantly increased tolbutamide-stimulated insulin release in normal (Fig. 5) and hyperparathyroid animals. This is in contrast to the normal response to tolbutamide in hypophosphatemic, normocalcemic dogs. These data suggest that tolbutamide-stimulated insulin release is increased by hypercalcemia but not by hypophosphatemia. On the other hand, glucose-stimulated insulin responses were unaffected by hypercalcemia, but were increased by hypophosphatemia.

Interrelationships between plasma calcium levels and insulin release after various stimuli have been previously suggested (11-17). Calcium seems to play a critical role in the release of stored insulin (17). It has been clearly demonstrated that glucose-stimulated insulin release is blunted both in vivo by hypocalcemia (12, 15) and in vitro when pancreatic islets are incubated in low-calcium media (11, 13, 14, 16, 17). Grodsky (17) and Curry et al. (13) have demonstrated that glucose-stimulated insulin release is calcium dependent only at levels below 4 meq/liter, with no apparent further effect at higher levels. Furthermore, calcium has been shown to have no direct insulin-stimulating characteristics (13), and may in fact depress glucose-mediated insulin release in vitro in the hypercalcemic range (14).

On the other hand, previous investigators have clearly demonstrated that tolbutamide-stimulated insulin release is calcium dependent at all concentrations (13). Marked augmentation in insulin release from a perfused isolated

pancreas occurs with tolbutamide stimulation if calcium levels in the perfusate are increased (13). Also, previous investigators have demonstrated that calcium infusions in normal volunteers will augment tolbutamide-stimulated insulin release, but will have no effect on glucose-stimulated insulin release (1). Thus it appears that glucose-stimulated insulin release is dependent upon and may be modulated by calcium within the physiologic range, but only tolbutamide-stimulated insulin release can be further increased by hypercalcemic states. The results of the present studies agree with these previous observations, and suggest that the mechanisms of tolbutamide- and glucose-stimulated insulin release may be functionally separate.

Acute PTH administration had no direct effect on glucose or tolbutamide-stimulated insulin response or  $K_d$  values in normal or hypophosphatemic dogs. Furthermore, in the dogs with severe secondary hyperparathyroidism, no change in glucose  $K_d$  or insulin responses to glucose or tolbutamide testing were noted. These data imply that PTH has no apparent direct effect on either glucose or tolbutamide-stimulated insulin release or on peripheral  $K_d$  in these animals. In the hyperparathyroid dogs, after the excessive phosphate was eliminated from the diet and plasma phosphorus levels fell, there was a greater increase in insulin response to glucose administration, despite a significant fall in plasma PTH levels (Fig. 5). This represents further evidence that phosphorus may play a critical role in the relative hyperinsulinemia and mild glucose intolerance of primary hyperparathyroidism.

The exact mechanisms by which plasma phosphate levels affect insulin release and glucose utilization are not clear. Previous investigators have demonstrated a relationship between phosphate and glucose uptakes (20-25). Furthermore, the depression of plasma phosphate after glucose administration is potentiated by insulin (26). Previous studies have demonstrated that glucose uptake by the perfused dog hind limb is associated with a fall in phosphate concentration in the perfusate (25). In vitro studies utilizing the intact rat diaphragm have shown that insulin administration will augment phosphate uptake even when glucose is not present in the incubating medium (27). Also, incubation of isolated rat muscle in high-phosphate medium will lead to increased basal and insulin-stimulated glucose uptake (28). Although omission of phosphate from the medium will not inhibit this uptake (29, 30), these studies have not been repeated in muscles from chronically hypophosphatemic animals.

Several mechanisms may be responsible for the impaired rate of glucose utilization seen in the hypophosphatemic animals. It is possible that hypophosphatemia induced by a very low phosphorus intake over a pro-



longed period significantly reduces intracellular concentrations of inorganic phosphorus, leading to reduced glucose uptake by peripheral muscle. The reduced uptake may reflect altered glucose phosphorylation, shown to influence the rate of glucose uptake by rat hemidiaphragm (31, 32). It is also possible that hypophosphatemia may alter phospholipid content of cell membranes, causing decreased uptake. For example, LeFevre et al. (33) have demonstrated that cell membrane phospholipid is critical in the transport process of all monosaccharides. Recently, phosphate depletion has been shown to lead to intracellular alkalosis (19), which may cause an altered intracellular sodium:potassium ratio produced by the efflux of potassium. By so doing, however, one would expect augmented glucose uptake (34, 35). On the other hand, the effect of intracellular pH on glucose uptake remains to be examined.

Further studies are needed to determine the role of known insulin antagonists such as glucagon, growth hormone, and cortisol in the genesis of the mild glucose intolerance and insulin resistance of hypophosphatemia. This is especially relevant in view of the augmented rate of recovery from comparable hypoglycemia seen between 50 and 90 min during the insulin tolerance tests (Fig. 4) in hypophosphatemic animals. This phase of recovery from hypoglycemia seems most likely related to factors that augment hepatic gluconeogenesis (36).

Hypophosphatemia may occur in many other disease states, including administration of phosphate-binding antacids (37), malnutrition (38, 39), and diabetic ketoacidosis. The abnormal insulin response and glucose intolerance noted in these conditions may in part reflect depressed plasma and tissue phosphorus levels. It seems important, when evaluating patients with abnormal glucose tolerance or hyperinsulinemia, to determine plasma phosphorus levels and, if hypophosphatemia is present, to correct it by dietary supplementation before interpretation of glucose disappearance rates or insulin responsiveness to a glucose challenge.

#### ACKNOWLEDGMENTS

The authors wish to express their appreciation to Sue King, Claire Pederson, and Steve Walker for their excellent technical assistance; and to Colleen Ahmad for her assistance in the preparation of this manuscript.

This investigation was supported by National Institute of Health grants AM-09976, AM-05248, and HD-06355.

#### REFERENCES

- Kim, H., R. K. Kalkhoff, N. V. Costrini, J. M. Cerletty, and M. Jacobson. 1971. Plasma insulin disturbances in primary hyperparathyroidism. *J. Clin. Invest.* **50**: 2596-2605.
- Yasuda, K., Y. Hurukawa, M. Okuyama, M. Kikuchi, and K. Yoshinaga. 1975. Glucose tolerance and insulin secretion in parathyroid disorders. Effect of serum calcium on insulin release. *N. Engl. J. Med.* **292**: 501-505.
- Mallette, L. E., J. P. Bilezikian, D. A. Heath, and G. D. Aurbach. 1974. Primary hyperparathyroidism: clinical and biochemical features. *Medicine (Baltimore)*. **53**: 127-146.
- Amend, W. J. C., Jr., S. M. Steinberg, E. G. Lowrie, J. M. Lazarus, J. S. Soeldner, C. L. Hampers, and J. P. Merrill. 1975. The influence of serum calcium and parathyroid hormone upon glucose metabolism in uremia. *J. Lab. Clin. Med.* **86**: 435-444.
- Harter, H. R., J. Santiago, E. Slatopolsky, and S. Klahr. 1975. Relative roles of phosphate, calcium and parathyroid hormone on glucose mediated insulin secretion. *Clin. Res.* **23**: 499A. (Abstr.)
- Harter, H. R., J. Santiago, A. Mercado, A. Pagliara, and S. Klahr. 1974. On the pathogenesis of the hyperinsulinemia of primary hyperparathyroidism. *Clin. Res.* **22**: 471A. (Abstr.)
- Olefsky, J., T. Batchelder, J. W. Farquhar, and G. M. Reaven. 1973. Dissociation of the plasma insulin response from blood glucose concentration during glucose infusions in normal dogs. *Metab. Clin. Exp.* **22**: 1277-1286.
- LeFlamme, G. H., and J. Jowsey. 1972. Bone and soft tissue changes with oral phosphate supplements. *J. Clin. Invest.* **51**: 2834-2840.
- Harter, H. R., A. Mercado, W. E. Rutherford, H. Rodriguez, E. Slatopolsky, and S. Klahr. 1974. Effects of phosphate depletion and parathyroid hormone on renal glucose reabsorption. *Am. J. Physiol.* **227**: 1422-1427.
- Hruska, K. A., R. Kopelman, W. E. Rutherford, S. Klahr, and E. Slatopolsky. 1975. Metabolism of immunoreactive parathyroid hormone in the dog. The role of the kidney and the effects of chronic renal disease. *J. Clin. Invest.* **56**: 39-48.
- Malaisse, W. J., G. Brisson, and F. Malaisse-Lagae. 1970. The stimulus-secretion coupling of glucose-induced insulin release. I. Interaction of epinephrine and alkaline earth cations. *J. Lab. Clin. Med.* **76**: 895-902.
- Littlelike, E. T., D. A. Witzel, and S. C. Whipp. 1968. Insulin: Evidence for inhibition of release in spontaneous hypocalcemia. *Proc. Soc. Exp. Biol. Med.* **129**: 135-139.
- Curry, D. L., L. L. Bennett, and G. M. Grodsky. 1968. Requirement for calcium ion in insulin secretion by perfused rat pancreas. *Am. J. Physiol.* **214**: 174-178.
- Milner, R. D. G., and C. N. Hales. 1967. The role of calcium and magnesium in insulin secretion from rabbit pancreas studied in vitro. *Diabetologica*. **3**: 47-49.
- Laron, Z., and T. Rosenberg. 1970. Inhibition of insulin release and stimulation of growth hormone release by hypocalcemia in a boy. *Horm. Metab. Res.* **2**: 121-122.
- Grodsky, G. M., and L. L. Bennett. 1966. Cation requirements for insulin secretion in the isolated perfused pancreas. *Diabetes*. **15**: 910-913.
- Grodsky, G. M. 1972. A threshold distribution hypothesis for packet storage of insulin. II. Effect of calcium. *Diabetes* **21** (Suppl.): 584-593.
- Lindall, A., R. Carmena, S. Cohen, and C. Comty. 1971. Insulin hypersecretion in patients on chronic hemodialysis. Role of parathyroids. *J. Clin. Endocrinol.* **32**: 653-658.
- Gold, L. W., S. G. Massry, A. I. Arieff, and J. W. Coburn. 1973. Renal bicarbonate wasting during phosphate depletion. A possible cause of altered acid-base

- homeostasis in hyperparathyroidism. *J. Clin. Invest.* **52**: 2556-2562.
20. Martini, P. F., G. Piancino, and A. Modica. 1967. Changes in phosphorus clearance determined by the administration of glucose in diseases of the parathyroids and in other pathological conditions. *Acta Endocrinol.* **54**: 629-636.
  21. Ginsburg, J. M. 1972. Effect of glucose and free fatty acid on phosphate transport in dog kidney. *Am. J. Physiol.* **222**: 1153-1160.
  22. Huffman, E. R., C. J. Hlad, Jr., N. E. Whipple and H. Elrick. 1958. The influence of blood glucose on the renal clearance of phosphate. *J. Clin. Invest.* **37**: 369-379.
  23. Halver, B. 1966. The effect of parathyroid hormone on the tubular reabsorption of glucose. *Acta Med. Scand.* **179**: 427-432.
  24. Harrop, G. A., Jr., and E. M. Benedict. 1924. The participation of inorganic substances in carbohydrate metabolism. *J. Biol. Chem.* **59**: 683-697.
  25. Pollock, H., R. F. Millet, H. E. Essex, F. C. Mann, and J. L. Bollman. 1934. Serum phosphate changes induced by injections of glucose into dogs under various conditions. *Am. J. Physiol.* **110**: 117-122.
  26. Briggs, A. P., I. Koechig, E. A. Doisy, and C. J. Weber. 1924. Some changes in the composition of blood due to the injection of insulin. *J. Biol. Chem.* **58**: 721-730.
  27. Walaas, O., E. Walaas, and A. N. Wick. 1969. The stimulatory effect by insulin on the incorporation of <sup>32</sup>P radioactive inorganic phosphate into intracellular inorganic phosphate, adenine nucleotides and guanine nucleotides of the intact isolated rat diaphragm. *Diabetologica.* **5**: 79-87.
  28. Randle, P. J., and G. H. Smith. 1958. Regulation of glucose uptake by muscle. 2. The effects of insulin, anaerobiosis and cell poisons on the penetration of isolated rat diaphragm by sugars. *Biochem. J.* **70**: 501-508.
  29. Chaudry, I. H., and M. K. Gould. 1969. Kinetics of glucose uptake on isolated soleus muscle. *Biochem. Biophys. Acta.* **177**: 527-536.
  30. Gould, M. K., and I. H. Chaudry. 1970. The action of insulin on glucose uptake by isolated rat soleus muscle. I. Effect of cations. *Biochem. Biophys. Acta.* **215**: 249-257.
  31. Kipnis, D. M. 1958. Penetration and phosphorylation of 2-deoxyglucose in isolated rat diaphragm. *Fed. Proc.* **17**: 254. (Abstr.)
  32. Kipnis, D. M. 1959. Regulation of glucose uptake by muscle: functional significance of permeability and phosphorylating activity. *Ann. N. Y. Acad. Sci.* **82**: 355-365.
  33. LeFevre, P. G., K. I. Habich, H. S. Hess, and M. R. Hudson. 1964. Phospholipid-sugar complexes in relation to cell membrane monosaccharide transport. *Science (Wash. D. C.)*. **143**: 955-957.
  34. Kohn, P. G., and T. Clausen. 1972. The relationship between the transport of glucose and cations across cell membranes in isolated tissues. VII. The effects of extracellular Na<sup>+</sup> and K<sup>+</sup> on the transport of 3-O-methylglucose and glucose in rat soleus muscle. *Biochem. Biophys. Acta.* **255**: 798-814.
  35. Clausen, T. 1975. The effect of insulin on glucose transport in muscle cells. *Curr. Top. Membranes Trans.* **6**: 169-209.
  36. Garber, A. J., P. E. Cryer, J. V. Santiago, M. Haymond, A. S. Pagliara, and D. M. Kipnis. 1975. Substrate and hormonal responses to hypoglycemia in man. *Diabetes.* **24**(Suppl. 2): 412. (Abstr.)
  37. Lotz, M., E. Zismon, and F. C. Bartter. 1968. Evidence for a phosphorus-depletion syndrome in man. *N. Engl. J. Med.* **278**: 409-415.
  38. Wayburne, S. 1968. Malnutrition in Johannesburg. In *Calorie Deficiencies and Protein Deficiencies*. R. A. McCance and E. M. Widdowson, editors. Little, Brown and Company, Boston, Mass. 7-21.
  39. Heard, C. R. C. 1968. Biochemical disturbances in experimental protein-calorie deficiency. In *Calorie Deficiencies and Protein Deficiencies*. R. A. McCance and E. M. Widdowson, editors. Little, Brown and Company, Boston, Mass. 249-256.