

The Effect of Chronic Insulin Therapy on Phosphate Metabolism in Diabetes Mellitus

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Summary. Plasma and urine phosphate concentrations were improved in 21 patients with diabetes mellitus during “optimal” metabolic control as compared with “suboptimal” control. During the “suboptimal” control phase the daily insulin dosage averaged 38 ± 22 (SD) U/day and the mean plasma glucose levels averaged 17.1 ± 1.8 mmol/l, while during the “optimal” control phase the daily insulin dosage averaged 84 ± 59 U/day and the mean plasma glucose level was 6.2 ± 1.4 mmol/l. The institution of rigid diabetic control over 4–10 days significantly raised serum phosphorus from 1.12 ± 0.16 to 1.26 ± 0.19 mmol/l ($p < 0.001$), and decreased urinary phosphorus excretion from 686 ± 125 to 588 ± 88 mg/day ($p < 0.001$). These changes were associated with significant reductions in urinary calcium, urinary glucose, plasma immunoreactive glucagon and serum parathyroid hormone. The diminution in urinary phosphorus loss may have been due to diminished glycosuria but equally could have been influenced by a direct action of insulin on the renal tubule or suppression of glucagon and parathyroid hormone secretion. Under the conditions of this study, reduced urinary phosphorus may have been sufficient to cause a rise in serum phosphorus despite the known effects of insulin on the cellular influx of phosphorus.

Key words: Diabetes mellitus, insulin, phosphorus, parathyroid hormone, glucagon

It is commonly believed that the administration of insulin lowers the circulating concentration of phosphorus by promoting cellular influx from the extracellular fluid [1, 2]. This effect is particularly apparent during the treatment of diabetic ketoacidosis [2]. However, this action of insulin may be opposed by an effect of insulin on the renal handling of phosphorus, since DeFronzo et al. have shown that the hormone may directly augment the renal tubular

reabsorption of phosphorus [3]. Moreover, insulin treatment may secondarily retard phosphorus excretion by suppressing parathyroid function. In a preliminary study [4], we have demonstrated that severe glycosuria in poorly controlled diabetic subjects may be associated with hypercalciuria and possibly with secondary hyperparathyroidism. The institution of rigid diabetic control with insulin produced a correction of the “renal leak” of calcium and a decline in urinary cyclic AMP [5]. Thus, these appear to be two opposing actions of insulin on plasma phosphate, one serving to lower its concentration by transfer into cells, the other tending to raise plasma levels by diminishing renal losses. The purpose of the present study was to examine the effects of improved diabetic control on plasma phosphate, calcium, parathyroid hormone levels and the urinary excretion of calcium and phosphorus.

Methods and Materials

Twenty-one patients with diabetes mellitus were studied; their clinical characteristics are listed in Table 1. All patients had previously been treated with insulin. All had an endogenous creatinine clearance greater than 80 ml/min. None had a history of pathological skeletal fracture, renal stones, or peptic ulcer disease. All drugs except insulin were withheld during the study. The study was approved by the Human Research Review Committee of the University of Texas Health Science Center at Dallas. Each patient gave informed consent.

The study was conducted at the General Clinical Research Center at Parkland Memorial Hospital for approximately 25 days while the patients consumed a constant metabolic diet, with a daily composition of 400 mg calcium, 800 mg phosphorus, and 100 mol sodium. Two-sevenths of the total daily calories were given at each meal and one-seventh as a bedtime snack. Caloric intake was sufficient to maintain stable body weight during the study.

The investigation was performed in two phases, an initial phase representing suboptimal insulin therapy with poor control of diabetes, and a subsequent phase of optimal insulin therapy with satisfactory diabetic control. Each phase consisted of 4–7 days of stabilization followed by three study days. During the suboptimal treatment period all patients were hyperglycaemic and had gly-

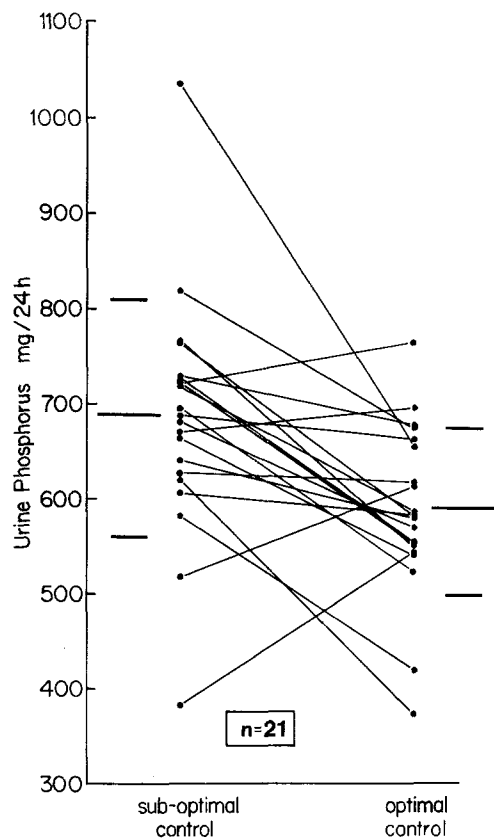


Fig. 1. The effect of chronic insulin therapy on urinary phosphorus excretion in 21 diabetic patients. The horizontal bars represent mean \pm SD

cosuria but none had hyperketonaemia or ketonuria. Plasma bicarbonate was normal in all patients. During the suboptimal treatment phase, all patients were treated with an arbitrarily selected dose of insulin, designed to maintain plasma glucose at approximately 17 mmol/l. After completion of the suboptimal phase, the insulin dose was progressively increased in an attempt to normalise the plasma glucose concentration (<8.3 mmol/l). Insulin was administered as a combination of neutral protamine Hagedorn (NPH) and regular (soluble) insulin 30 min before breakfast and dinner. Each patient's daily dose of insulin was determined by the glycaemic response to the previous day's insulin dose.

During each of the three days of the study period, daily 24 h urine collections were made for glucose, calcium, phosphorus, and creatinine estimations. Diabetic regulation was assessed on the basis of plasma glucose determinations made from blood samples obtained five times daily (before each meal and at 03.00 h) from an indwelling forearm vein cannula. Plasma samples for measurement of immunoreactive glucagon were obtained simultaneously. The mean plasma glucose and immunoreactive glucagon concentrations for each study period (suboptimal and optimal) were computed from the 15 measurements made during each 3-day period. Fasting morning blood samples were obtained at 08.00 h at the end of each day's urine collection, for the measurement of calcium, phosphorus and creatinine, and for parathyroid hormone by immunoassay (iPTH).

The endogenous phosphate clearance and creatinine clearance were calculated for each 24 h period. Tubular reabsorption of phosphorus represented $1 - (\text{phosphorus clearance} / \text{creatinine clear-})$

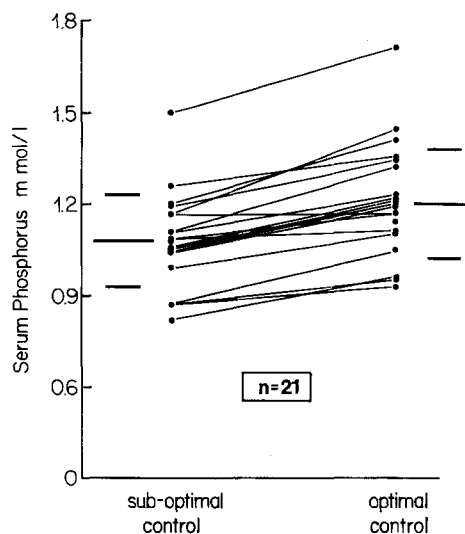


Fig. 2. The effect of insulin therapy on serum phosphorus concentration in 21 diabetic patients. The horizontal bars represent mean \pm SD

ance). The renal threshold phosphorus concentration was calculated from the nomogram of Walton and Bijvoet utilising tubular reabsorption of phosphorus and corresponding serum phosphorus [6].

Glucose in plasma and urine was measured by the glucose oxidase method on the Beckman glucose analyzer. Serum calcium and phosphorus were measured with an autoanalyser, urinary calcium was measured by atomic absorption spectrophotometry, and urinary phosphorus by the method of Fiske and Subbarow [7]. Specimens for immunoreactive glucagon were collected in chilled tubes containing EDTA 12 mg and 1 ml of Trasylol (500 kallikrein inhibitor units/ml of blood) and centrifuged promptly at 4 °C. Plasma was separated and stored at -20 °C until the time of hormone assay. Plasma immunoreactive glucagon was determined by a previously described method using antibody 30K [8]. This assay can recognize a difference of 10 pg/ml with 95% confidence. Serum iPTH was measured by a modification of a previously described radioimmunoassay [9, 10]. This assay used an antiserum (Iso-Tex Diagnostic, Friendswood, Texas) directed primarily against the carboxy-terminal region of bovine PTH. Bovine PTH, used as a tracer, was iodinated as previously described. Human PTH (a generous gift of Dr. B. Roos) was used as the standard. The detection limit of this assay was 0.4 ng/ml, with the normal upper limit being 1.9 ng/ml. For each of the various parameters an average value was computed for each 3-day study period, and the values during the suboptimal and optimal insulin treatment periods were compared using Student's t-test for paired samples. Values in results are presented as mean \pm SD.

Results

During the period of suboptimal diabetic control with insulin, the mean daily dose of insulin was 39 ± 5 units/day. The mean plasma glucose concentration was 17.1 ± 1.8 mmol/l and glucose excretion averaged 114 ± 44 g/day. During the period of optimal diabetic control, the insulin dose averaged 79 ± 10 units/day. The mean plasma glucose concentration

Table 1. Clinical characteristics of diabetic patients

Patient	Age (years)	Sex	% Ideal body weight	Duration of diabetes (years)	Insulin dosage			
					"suboptimal" period (U/day)		"optimal" period (U/day)	
					am	pm	am	pm
1	36	M	102	2	33	16	34	19
2	16	F	103	7	35	—	67	27
3	22	M	90	5	38	—	48	20
4	32	M	102	4	38	—	42	25
5	18	M	114	10	40	—	38	25
6	17	M	85	2	25	—	37	11
7	34	F	92	11	35	—	31	16
8	16	F	91	4	42	—	52	18
9	22	F	109	6	35	10	48	17
10	34	F	97	9	32	—	45	10
11	17	F	112	6	30	—	49	10
12	28	F	109	5	30	—	40	30
13	37	F	96	15	30	—	22	16
14	30	M	94	25	33	—	30	19
15	19	M	86	5	28	—	42	21
16	23	F	122	5	25	—	50	24
17	41	F	166	3	50	—	105	45
18	56	M	160	2	25	—	90	80
19	64	F	139	29	5	—	24	12
20	48	F	244	16	100	25	150	70
21	23	F	135	2	30	—	50	15

was 6.2 ± 1.4 mmol/l ($p < 0.001$) and glucose excretion fell to 4 ± 5 g/day ($p < 0.001$).

During the suboptimal insulin treatment fasting serum phosphorus for the whole group averaged 1.12 ± 0.16 mmol/l and urinary phosphorus excretion averaged 686 ± 125 mg/day. Improved diabetic control resulted in a significant increase in the serum phosphorus concentration to 1.26 ± 0.19 mmol/l ($p < 0.001$). Urinary phosphorus fell significantly to 588 ± 88 mg/day ($p < 0.001$; Fig. 1). Serum phosphorus obtained before breakfast rose in all patients with optimal insulin therapy (Fig. 2). Urinary phosphorus fell in 17 out of 21 patients. The 24 h phosphorus clearance fell from 13.7 ± 3.9 mg/ml in the suboptimal control period to 10.4 ± 2.5 mg/ml ($p < 0.001$).

There was a significant increase in both tubular reabsorption of phosphorus (from 87 ± 3 to $90 \pm 3\%$; $p < 0.001$) and renal threshold phosphorus concentration (from 1.08 ± 0.22 to 1.29 ± 0.21 mmol/l; $p < 0.01$) with the institution of rigid diabetic control. Urinary calcium excretion averaged 169 ± 65 mg/24 h during the suboptimal insulin treatment period and fell significantly to 118 ± 58 mg/24 h with optimal insulin treatment ($p < 0.001$). Serum calcium concentration was unchanged by insulin therapy.

During the suboptimal treatment phase, mean plasma immunoreactive glucagon averaged $166 \pm$

29 pg/ml. With the institution of optimal diabetic control, there was a significant reduction in the mean plasma immunoreactive glucagon to 131 ± 27 pg/ml ($p < 0.01$). Values for serum iPTH were not elevated during the suboptimal control phase, averaging 0.80 ± 0.38 ng/ml. During optimal insulin treatment, serum iPTH declined slightly to 0.65 ± 0.30 ng/ml; this change was significant ($p < 0.01$).

Discussion

The current study clearly shows that the institution of optimal diabetic control over 4–10 days increases the serum concentration of phosphorus and decreases urinary phosphorus excretion. This decrease in urinary phosphorus is probably the result of several factors. First, hyperphosphaturia has long been known to occur with glycosuria and seems to be dependent upon the presence of glucose in the urine. It does not occur with an osmotic diuresis caused by mannitol [11]. The elimination of glycosuria by optimum insulin therapy would tend to attenuate hyperphosphaturia. The second possible explanation is a diminution of the hyperglucagonaemia which is present in poorly controlled diabetes [12]. In the rat, glucagon has been shown to have a direct phosphaturic effect on the renal tubule [13]. In our study plasma glucagon concentration fell with the institu-

tion of good diabetic control, as shown previously [14]. The resulting decrease in circulating glucagon could have diminished urinary phosphorus excretion.

The third possible explanation involves secondary hyperparathyroidism. During suboptimal insulin therapy, the renal "leak" of calcium associated with glycosuria could have stimulated parathyroid function, and optimal insulin therapy could have reversed the process. This suggestion is supported by our previous finding [4] of reduced renal excretion of calcium and cyclic AMP during optimal therapy. In the present study, a significant decline in urinary calcium was also shown. Moreover, serum iPTH declined significantly during optimal insulin treatment. It is unlikely that this explanation could have accounted for all of the reduction in urinary phosphorus, in so far as the change in serum iPTH levels was small.

Finally, decreased phosphorus excretion might have resulted from a direct effect of insulin on the renal tubule. DeFronzo et al. [3] have shown in human subjects that the infusion of insulin sufficient to maintain plasma insulin concentration within the normal range, significantly lowered urinary phosphorus. The diminution in urinary phosphorus excretion, produced by direct and indirect actions of insulin described above, must be principally renal in origin, since phosphorus clearance decreased and tubular reabsorption of phosphorus and renal threshold phosphorus concentration increased significantly, despite a rise in the filtered load of phosphorus. The results suggest that this reduced renal excretion of phosphorus may have caused the increase in serum phosphorus concentration.

The finding of the increased circulating concentration of phosphorus encountered during optimal insulin therapy is paradoxical since insulin itself has been shown to cause a shift of phosphorus from the extracellular space to the intracellular space [1, 2]. If this action of insulin on cellular flux of phosphorus were the only mechanism, one would have anticipated a fall in serum phosphorus concentration. The data indicate that the renal effects of insulin with respect to phosphorus transport may have predominated over its action on cellular phosphorus fluxes under the particular conditions of this experiment.

In conclusion, the balance between the action of insulin on renal handling of phosphorus and on cellular flux of phosphorus probably determines directional changes in serum phosphorus concentration. Under steady-state conditions, optimal insulin treatment increases serum phosphorus by reducing phosphorus excretion. It is of interest that Gertner et al. [5] reached essentially identical conclusions on the basis of studies in a smaller number of diabetic patients.

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