Vitamin D Deficiency Inhibits Pancreatic Secretion of Insulin

Abstract. *The effect of a vitamin D deficiency on insulin and glucagon release was determined in the isolated perfused rat pancreas by radioimmunoassay of the secreted proteins. During a 30-minute period of perfusion with glucose and arginine, pancreases from vitamin D–deficient rats exhibited a 48 percent reduction in insulin secretion compared to that for pancreases from vitamin D–deficient rats that had been replenished with vitamin D. Vitamin D status had no effect on pancreatic glucagon secretion. This result, along with the previously demonstrated presence in the pancreas of a vitamin D–dependent calcium-binding protein and cytosol receptor for the hormonal form of vitamin D, 1,25-dihydroxyvitamin D_3, indicates an important role for vitamin D in the endocrine functioning of the pancreas.*

Vitamin D_3 is essential in higher animals to effect normal calcium and phosphorus homeostasis (*1*). This is accomplished by an endocrine system that regulates the sequential metabolism of vitamin D_3 by the liver and kidney into its two principal biologically active metabolites, 1,25-dihydroxyvitamin D_3 [1,25-$(OH)_2D_3$] (*1*, *2*) and 24,25-dihydroxyvitamin D_3 [24,25-$(OH)_2D_3$] (*3*). The best documented biological actions of vitamin D_3 and its most active metabolite, 1,25$(OH)_2D_3$, are the stimulation of intestinal calcium absorption and the mobilization of bone calcium (*1*).

The mode of action of 1,25-$(OH)_2D_3$ in stimulating intestinal calcium absorption is believed to be analogous to that of other steroid hormones (*1*, *2*). Biochemical evidence supports the existence of a cytosol protein receptor for 1,25-$(OH)_2D_3$ in the intestine of the chick (*4*), rat (*5*), and man (*6*). This 1,25-$(OH)_2D_3$ steroid-receptor complex migrates to the nucleus of the cell and stimulates the biosynthesis of a number of messenger RNA molecules that code for proteins related to the generation of the biological response—including a calcium-binding protein (CaBP) (*7*). It is not yet clear whether CaBP in the intestine has an obligatory role in effecting the transepithelial transport of calcium ions across the cell or whether it is present for other reasons, which might include "protection" of the cell against the deleterious effects of high concentrations of intracellular Ca^{2+} occurring as a consequence of vitamin D–mediated intestinal calcium absorption. However, the presence of CaBP is totally dependent on the presence of 1,25-$(OH)_2D_3$ (*7*); the amount of CaBP in the chick intestine is exactly proportional to the amount of 1,25-$(OH)_2D_3$ localized in the intestinal mucosa (*8*).

By a sensitive and specific radioimmunoassay (*9*) with antibodies against highly purified chick intestinal CaBP (*10*), we have detected vitamin D–dependent CaBP in the chick bone (*11*), kidney, intestine, and pancreas (*9*). The actions of vitamin D_3 and 1,25-$(OH)_2D_3$ (*1*) and of receptors for 1,25-$(OH)_2D_3$ have been documented in the parathyroid gland (*12*), bone (*13*), and kidney (*14*), but the presence of a vitamin D–dependent CaBP in the pancreas was unexpected. We have, however, reported the presence in the chick pancreas of a protein receptor for 1,25-$(OH)_2D_3$ (*14*) homologous to the 1,25-$(OH)_2D_3$ receptors previously described in the chick intestine (*4*), parathyroid gland (*12*), and bone (*13*).

The presence in the chick pancreas of cytosol receptors for 1,25-$(OH)_2D_3$ as well as CaBP is evidence of a hitherto unappreciated role of vitamin D and its metabolites in pancreatic endocrine and exocrine function. We now describe our initial efforts to determine whether vitamin D deficiency has any effect on the endocrine function of the rat pancreas.

Weanling rats (Holzman, Madison, Wis.) were raised on a standard vitamin D–deficient nonrachitogenic diet (*15*) in a room devoid of ultraviolet light. After 12 weeks the rats were shipped in light-restricted cages to San Francisco, where they were fed the same vitamin D–deficient diet and were given free access to water.

At approximately 72, 48, and 24 hours prior to perfusion, the restrained unanesthetized rats were given either 0.2 ml of ethanol (95 percent) (D-deficient animals) or 0.2 ml of a solution (95 percent ethanol and 1,2-propanediol, 1:1 by volume) containing 200 I.U. (5.0 μg) of vitamin D_3 (D-replete animals). Just before the pancreas was removed, arterial blood was collected, heparinized, chilled in ice, centrifuged, and stored at −20°C until it was assayed for calcium, phosphorus, magnesium, and 25-$(OH)D_3$. The serum concentrations of calcium, phosphorus, and 25-$(OH)D_3$ reported in Table 1 are typical for vitamin D–deficient and replete rats (*1*). Although body weights and pancreas weights tended to be slightly greater in D-replete than in D-deficient animals, the differences were not statistically significant. The secretion of insulin and glucagon in vitro was assessed by the technique of Grodsky and Fanska (*16*). To exclude extrapancreatic sources of glucagon and to conserve the albumin in the perfusate, we used a modified preparation (*17*) in which the stomach, spleen, and most of the duodenum were removed. Briefly, the pancreas and proximal duodenum were perfused in vitro with a modified Krebs-Ringer biocarbonate buffer (*p*H 7.4) with the following millimolar composition: Na^+, 142; K^+, 5.9; Ca^{2+}, 2.35; Mg^{2+}, 1.22; $H_2PO_4^-$, 1.5; HCO_3^-, 29; Cl^-, 119, and SO_4^{2+}, 1.2. The

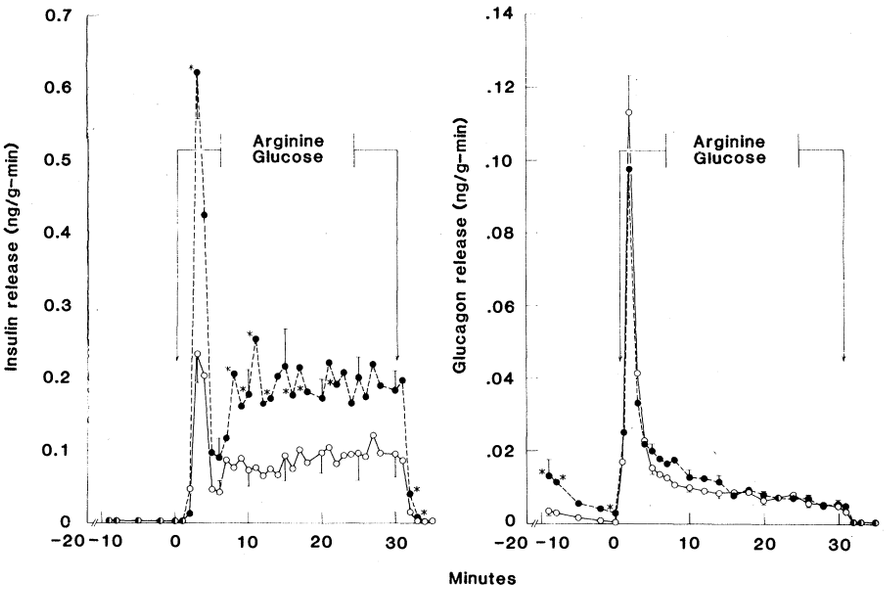

Fig. 1. Effect of a vitamin D deficiency on glucose-plus-arginine–stimulated insulin and glucagon release from the isolated perfused rat pancreas; (○) D-deficient (N = 7) and (●) D-replete (N = 5) animals.

0036-8075/80/0815-0823$00.50/0

Table 1. Parameters for D-deficient and D-replete rats. Serum Ca^{2+} and Mg^{2+} were determined by atomic absorption analysis; serum P was determined by the Fiske-Subbarow method; and serum 25-(OH)D levels were determined by a steroid competition assay (*16*). Data are expressed as means ± standard error of the mean.

Group	*N*	Serum Mg^{2+} (mg/100 ml)	Serum Ca^{2+} (mg/100 ml)	Serum P (mg/100 ml)	Serum 25-(OH)D (ng/ml)	Body weight (g)	Pancreas weight (g wet weight)
D-deficient	7	4.9 ± 0.5	4.1 ± 0.3	9.5 ± 0.7	≤ 0.5	278 ± 9	1.28 ± 0.05
D-replete	4–5	4.8 ± 0.4	5.7 ± 0.3	7.4 ± 0.5	26.1*	315 ± 22	1.61 ± 0.22
P†		N.S.	< .005	N.S.		N.S.	N.S.

*Data are given as the mean of duplicate determinations on a pooled sample from four rats. †Two-tailed Student's *t*-test; N.S., no significant difference.

perfusate also contained 1 percent human serum albumin (Armour, Kankakee, Ill.) and 3 percent Dextran T-40 (Pharmacia, Piscataway, Md.). The perfusate was oxygenated (95 percent O_2 and 5 percent CO_2), warmed to 37°C, and introduced into the celiac artery, and then the complete effluent was collected from the portal vein every minute for 55 to 60 minutes in chilled tubes containing 12 mg of EDTA. The lag time between the perfusate leaving the pancreas and reaching the fraction collector was about 30 seconds (2.0 ml). Pancreases were perfused at least 10 minutes before any samples were collected for hormone assay. The flow rate was maintained at 4 ml/min, and the arterial pressure was 37 to 50 mm-Hg. Glucose (5.5 m*M*) and arginine hydrochloride (10 m*M*) were dissolved in the perfusate and infused at the appropriate times at 0.1 ml/min through a sidearm syringe.

Immunoreactive insulin was measured by a solid-phase modification (*18*) of a single-antibody assay (*19*), with rat insulin used as a standard. Immunoreactive glucagon was measured by a dextran-charcoal method modified (*20*) from that of Unger and Eisentraut (*21*); Unger 30K antiserum was used. Hormone release is expressed as nanograms per gram of body weight per minute to eliminate the variation in body weight between the two groups.

In response to combined stimulation with glucose and arginine, a typical biphasic pattern of insulin release (*20*) was seen for the D-replete animals (Fig. 1). This response consisted of an early burst of insulin release (peak 3 minutes after start of stimulus), a fall in the insulin release to a nadir at 6 minutes, and then a fairly consistent second phase until the end of the stimulation at 30 minutes. In the D-deficient pancreases, the first phase (0 to 6 minutes) and second phase (6 to 30 minutes) of insulin release were only 49 and 47 percent, respectively, of the release from the D-replete pancreases ($P < .05$, Fig. 2). The glucagon response (*20*) to the combined infusion of glucose and arginine was biphasic (first spike plus slowly declining second phase) and was not different in the D-deficient and D-replete animals ($P > .60$).

Our results clearly indicate that in a vitamin D–deficient state there is an impaired secretion of insulin by the rat pancreas in response to the glucose-arginine secretagogue. In a D-deficient animal the serum Ca^{2+} concentration is only approximately 40 to 45 percent of the serum Ca^{2+} concentration in a normal animal [normal, 9 to 10 mg/100 ml (*15*)]. During the 72 hours of vitamin D repletion (Table 1), the serum Ca^{2+} concentration increased from 4.05 to 5.7 mg/100 ml ($P < .005$) while the Mg^{2+} concentration remained unchanged. Since insulin release in vitro is dependent on acute changes in Ca^{2+} or in the ratio of Ca^{2+} to Mg^{2+} (*22, 23*), the improved release in the D-replete animals may have resulted from the increased Ca^{2+} rather than from the effect of vitamin D or its metabolites. However, it is emphasized that the in vitro pancreatic experiments with depleted and repleted animals were performed with the same (normal) Ca^{2+} concentration; any effect of the prolonged increases in Ca^{2+} in vivo, particularly when followed by normalization in vitro, is unknown. Glucagon secretion is also Ca^{2+} dependent (*18*), but secretion of this hormone was unaffected, an indication that the glucagon-producing cells are less sensitive than the insulin-producing cells to the changes invoked by vitamin D.

We know of no explicit demonstration of vitamin D in pancreatic function prior to the present study and our recent demonstration of the presence of a vitamin D–dependent CaBP in the pancreas, as well as of a cytosol receptor for 1,25-$(OH)_2D_3$. There were, however, several reports of the deleterious effects of diabetes on vitamin D functioning and calcium homeostasis. The existence of a relation between pancreatic function and calcium metabolism was known from clinical studies in which patients with both juvenile- and adult-onset diabetes were found to have significant bone disease (*24, 25*). Also, Schneider *et al.* (*26*) found that duodenal calcium absorption and levels of intestinal vitamin D–dependent CaBP are depressed in diabetic rats. Treatment with 1,25-$(OH)_2D_3$, but not with 25-$(OH)D_3$ or vitamin D_3, restores duodenal calcium absorption and CaBP to normal (*27*). In the diabetic rat, the serum concentration is normal for 25-$(OH)D_3$, but is depressed to one-eighth normal for 1,25-$(OH)_2D_3$; serum concentration of 1,25-$(OH)_2D_3$ is restored to

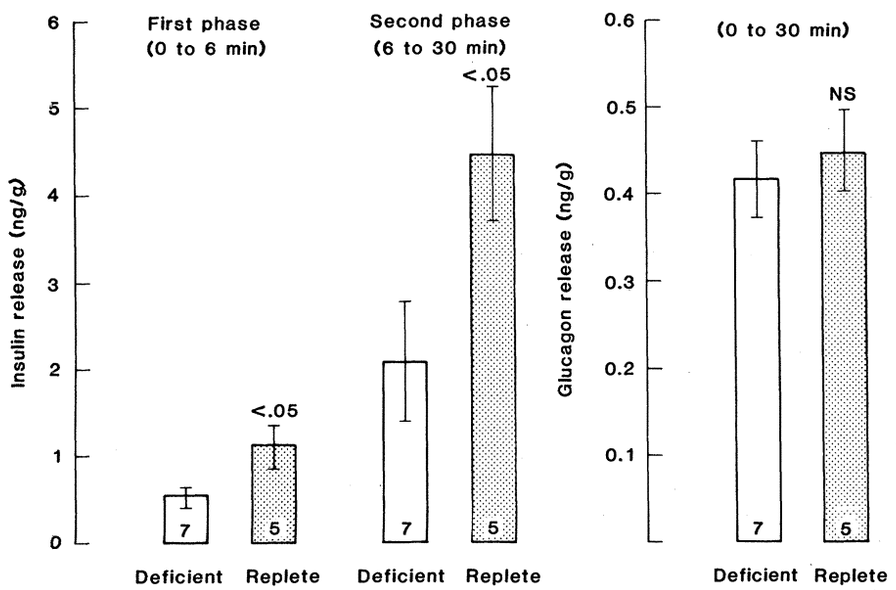

Fig. 2. Quantification of the insulin and glucagon responses in vitro from the pancreases of D-deficient and D-replete rats.

control levels by insulin treatment (*28*).

The vitamin D endocrine system has a wide variety of physiologically relevant interactions with other hormones besides insulin; these include parathyroid hormone, calcitonin, growth hormone, prolactin, glucocorticoids, and estrogens (*1*). Our results documenting a vitamin D-pancreas interaction are not surprising in view of our expanding understanding of the breadth of this secosteroid action in calcium homeostasis. The role of CaBP in cellular calcium metabolism may be relevant in elucidating the physiological significance as well as the biochemical mode of action of vitamin D in the pancreatic secretion of insulin.

ANTHONY W. NORMAN
Department of Biochemistry, University of California, Riverside 92521

BARBARA J. FRANKEL
ANNELIESE M. HELDT
GERALD M. GRODSKY
Metabolic Research Unit, School of Medicine, University of California, San Francisco 94143

References and Notes

1. A. W. Norman, *Vitamin D: The Calcium Homeostatic Steroid Hormone* (Academic Press, New York, 1979).
2. _____ and F. P. Ross, *Life Sci.* **24**, 759 (1979); M. R. Haussler and T. McCain, *N. Engl. J. Med.* **297**, 974 and 1041 (1977); H. Schnoes and H. F. DeLuca, *Annu. Rev. Biochem.* **45**, 631 (1976).
3. H. L. Henry and A. W. Norman, *Science* **201**, 835 (1978); J. M. Canterbury, S. Lerman, A. J. Claflin, H. Henry, A. W. Norman, E. Reiss, *J. Clin. Invest.* **61**, 1375 (1978); H. L. Henry, A. N. Taylor, A. W. Norman, *J. Nutr.* **107**, 1918 (1977); A. W. Norman, H. L. Henry, H. Malluche, *Life Sci.* **27**, 229 (1980).
4. H. C. Tsai and A. W. Norman, *J. Biol. Chem.* **248**, 5967 (1973); W. R. Wecksler, W. H. Okamura, A. W. Norman, *J. Steroid Biochem.* **9**, 929 (1978); P. F. Brumbaugh and M. R. Haussler, *J. Biol. Chem.* **249**, 1251 (1974).
5. B. E. Kream and H. F. DeLuca, *Biochem. Biophys. Res. Commun.* **76**, 735 (1977); W. R. Wecksler, F. P. Ross, A. W. Norman, *J. Biol. Chem.* **254**, 9488 (1979).
6. W. R. Wecksler, R. S. Mason, A. W. Norman, *J. Clin. Endocrinol. Metab.* **48**, 715 (1979); W. R. Wecksler, F. P. Ross, R. S. Mason, A. W. Norman, *ibid.* **50**, 152 (1980).
7. A. N. Taylor and R. H. Wasserman, *Arch. Biochem. Biophys.* **119**, 536 (1967); R. L. Morrisey and R. H. Wasserman, *Am. J. Physiol.* **220**, 1509 (1971).
8. E. J. Friedlander, H. Henry, A. W. Norman, *J. Biol. Chem.* **252**, 8677 (1977).
9. S. Christakos, E. J. Friedlander, B. R. Frandsen, A. W. Norman, *Endocrinology* **104**, 1495 (1979).
10. E. J. Friedlander and A. W. Norman, *Methods Enzymol.* **67**, 504 (1980).
11. S. Christakos and A. W. Norman, *Science* **202**, 70 (1978).
12. P. F. Brumbaugh, M. R. Hughes, M. R. Haussler, *Proc. Natl. Acad. Sci. U.S.A.* **72**, 4871 (1975); W. R. Wecksler, H. L. Henry, A. W. Norman, *Arch. Biochem. Biophys.* **183**, 168 (1977); W. R. Wecksler and A. W. Norman, *J. Miner. Electrolyte Metab.* **1**, 99 (1978).
13. B. E. Kream, M. Jose, S. Yamada, H. F. DeLuca, *Science* **197**, 1086 (1977).
14. S. Christakos and A. W. Norman, *Biochem. Biophys. Res. Commun.* **89**, 56 (1979).
15. H. Steenbock and D. C. Herting, *J. Nutr.* **57**, 449 (1955).
16. G. M. Grodsky and R. E. Fanska, *Methods Enzymol.* **39**, 364 (1975).
17. _____, I. Lundquist, *Endocrinology* **97**, 573 (1975).
18. I. Lundquist, R. Fanska, G. M. Grodsky, *ibid.* **99**, 1304 (1976).
19. G. M. Grodsky and P. H. Forsham, *J. Clin. Invest.* **39**, 1070 (1960).
20. J. E. Gerich, M. A. Charles, G. M. Grodsky, *ibid.* **54**, 833 (1974).
21. R. H. Unger and A. M. Eisentraut, in *Hormones in Blood*, G. H. Grey and A. L. Bacharach, Eds. (Academic Press, New York, 1967), vol. 1, p. 83.
22. D. L. Curry, L. L. Bennett, G. M. Grodsky, *Endocrinology* **83**, 572 (1968).
23. L. L. Bennett, D. L. Curry, G. M. Grodsky, *ibid.* **85**, 594 (1969).
24. M. E. Levin, V. C. Boisseau, L. V. Avioli, *N. Engl. J. Med.* **294**, 241 (1976).
25. J. D. Ringe, F. Kuhlencordt, H. P. Kruse, *Am. J. Roentgenol.* **126**, 1300 (1976).
26. L. E. Schneider, D. H. Wilson, H. P. Schedl, *Nature (London)* **245**, 327 (1973).
27. L. E. Schneider, J. Omdahl, H. P. Schedl, *Endocrinology* **99**, 793 (1976).
28. L. E. Schneider, H. P. Schedl, T. McCain, M. R. Haussler, *Science* **196**, 1452 (1977).
29. This work was supported in Riverside by grants AM-09012 and AM-14750 and in San Francisco by grant AM-01410. We are indebted to Mary Ann Jones and Michele Manning for their technical assistance. All inquiries should be addressed to A.W.N.

8 November 1979; revised 24 March 1980

Factors Influencing the Inhibitory Effect of Selenium on Mice Inoculated with Ehrlich Ascites Tumor Cells

Abstract. *Selenium, administered to mice with Ehrlich ascites tumors, effectively limited tumor growth. The response was dependent on the chemical form and dose of selenium administered. At the doses administered, there were no detectable adverse effects to the host.*

Selenium occurs naturally in many foods. As early as 1957, selenium was considered an essential nutrient (*1*), yet not until 1979 was a recommended dietary allowance for this metallic element proposed. Recent epidemiological evidence shows a negative correlation between selenium intake and tumor incidence in man (*2–8*). Also, selenium has been shown to be capable of inhibiting chemically induced, transplantable, and spontaneous tumor growth (*9–12*). Numerous factors may influence the ability of selenium to retard tumor incidence and growth. The present studies were designed to determine the influence of the chemical form and concentration of various selenium compounds on the growth of transplantable Ehrlich ascites tumor cells (EATC).

The tumor cell line was maintained in our laboratory by transplantation into Swiss/ICR mice at 14-day intervals. On day 0 of the experimental period, ascites tumors were removed from a stock animal and diluted in Krebs-Ringer-phosphate (KRP) solution consisting of 0.085*M* Na_2HPO_4, 0.016*M* NaCl, 0.005*M* KCl, and 0.0013*M* $MgSO_4 \cdot 7H_2O$ (*p*H 7.4). The cells were counted with a hemocytometer, and their viability was estimated by trypan blue dye exclusion (*13*). Cell viabilities were at least 98 percent. Each inoculum contained approximately 5×10^5 living EATC.

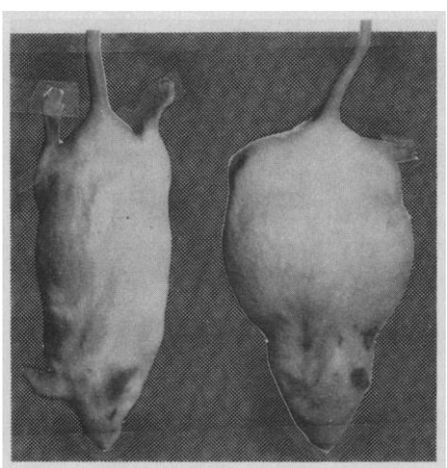

Fig. 1. Effects of treating or not treating EATC-inoculated mice with selenium. The mouse on the left received sodium selenite (1 μg per gram of body weight) and gained only 1.3 g in 21 days. The mouse on the right received KRP solution and gained 22.4 g.

Male Swiss/ICR mice weighing 20 to 22 g were used in the studies. They had free access to Purina mouse chow and distilled water and were housed in shoebox cages in groups of five. On day 0, the mice were inoculated with EATC and given intraperitoneal injections of KRP or a selenium compound dissolved in KRP. In general, each treatment was administered to ten EATC-inoculated mice and three noninoculated mice. The selenium (2.0 μg per gram of initial body weight) was administered as selenium dioxide, sodium selenite, sodium selenate, selenocystine, or selenomethionine. Additional injections followed on days 1, 3, 5, 7, 9, 12, 15, 18, and 21 (approximately 0.1 ml was administered per injection). The treatments were assigned at random.

Twenty-one days after being inoculated with EATC, half of the selenium-treated mice were killed by cervical dislocation and autopsied to determine the presence or absence of ascites tumors. The remaining EATC-treated mice were maintained without further selenium treatments for 21 more days and then were killed. On days 21 and 42, no tumors were visually evident in any of

0036-8075/80/0815-0825$00.50/0