# Somatostatin and Pancreatic Polypeptide Secretion

## Effects of Glucagon, Insulin, and Arginine

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**SUMMARY**

**The isolated perfused canine pancreas with duodenal exclusion was used to examine islet hormone output in response to arginine and exogenous glucagon and insulin. Exogenous glucagon (100 ng/ml) stimulated insulin and somatostatin secretion, which occurred in a biphasic pattern. The insulin response to glucagon was markedly enhanced by increased perfusate glucose, unlike the somatostatin response, which was little affected. The insulin and somatostatin responses were seen between 15 and 45 s after the glucagon stimulus. Pancreatic polypeptide secretion was uninfluenced by exogenous glucagon.**

**Biphasic release of glucagon, somatostatin, and pancreatic polypeptide was evoked by 10 mM arginine, the responses first being apparent within less than 30 s. Exogenous insulin (50 mU/ml) infused for 10 min had no statistically significant effect on glucagon, somatostatin, or pancreatic polypeptide secretion. This study suggests that these four islet hormones may all be involved in the dynamic mechanisms of nutrient metabolism. In addition, potential intra-islet paracrine effects are identified. DIABETES *28*:35–40, January 1979.**

Our understanding of islet physiology has been complicated in recent years by the identification of newly discovered peptides in discrete islet cell types. Somatostatin is contained in D cells[1] and has well-described inhibitory effects on both insulin and glucagon secretion[2,3] as well as on pancreatic polypeptide secretion,[4,5] but the function of this islet peptide is unknown. Pancreatic polypeptide is found in what is now known as the PP cell,[6] and even less is known about its actions and physiologic contributions. The development of radioimmunoassays for these two peptides has allowed preliminary studies of their secretory characteristics to be undertaken.[4,7–23]

To better understand islet function we must learn more about how secretory products of certain islet cells influence their neighboring cells. Although it has been shown that glucagon stimulates somatostatin secretion,[7,11,17,24] little is known about such details as timing of release, pattern of secretion, and the influence of varying glucose concentration on the process. Although the characteristics of pancreatic polypeptide secretion in vivo are being defined,[4,18–20,22,23] little data in vitro are available.[21,23] Therefore, the isolated perfused canine pancreas with duodenal exclusion was used to study the secretion of somatostatin and pancreatic polypeptide in response to arginine and exogenous insulin and glucagon.

This work was presented in part at the Annual Meeting of the American Federation for Clinical Research, Washington, D.C., May 1, 1977; and at the Annual Meeting of the Endocrine Society, Chicago, Ill., June 8, 1977.

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Accepted for publication 8 September 1978.

## METHODS

**Perfused canine pancreas preparation.** Conditioned German shepherd dogs that were fasted overnight were used, with isolation of the pancreas achieved by a modification of the method described by Iverson.[25] The duodenum was excluded by a clamp that prevents cross-contamination from duodenal hormones. The duration of surgery was approximately 2 h, and pancreas and perfusate were kept at 38°C. Perfusate flow rate was held constant at 20 ml/min, with not more than a 10% variation from this. The perfusate contained 4% dextran T70 (Pharmacia Fine Chemicals), 0.2% bovine serum albumin (Sigma), Krebs-Ringer bicarbonate buffer with electrolyte concentrations adjusted to those found in dog plasma, and fumarate, pyruvate, and glutamate, each at a concentration of 5 mM. Depending on the experiment, the perfusate glucose concentration was either 15, 88, 150, or 300 mg/dl. Exogenous porcine glucagon or insulin (both obtained from Lilly and Company, Indianapolis, Indiana) was introduced via a side-arm syringe to achieve a concentration of 100 ng/ml of

perfusate glucagon or 50 mU/ml of insulin, concentrations chosen because they approximate those theoretically present in islet interstitial fluid.[32,45] In experiments in which closely timed sample collections were required, samples were obtained during consecutive 15-s intervals; otherwise they were obtained during the first 15 s of a given minute. The infusion of glucagon, insulin, or arginine into the afferent tubing was begun 30 s before the initiation point shown on the graphs; and these secretagogues reached the collection tubes 50–55 s later (20–25 s after the graphed initiation point), as determined by the visual marker methylene blue. The dead space of the system, which varies slightly because of minor differences in pancreas size, is approximately 18 ml.

There was always an equilibration period of 40 min following surgery before experimental samples were obtained, and the experimental period was of a maximum duration of 180 min. The maximum number of experiments within any given perfusion was six for glucagon and two for arginine. There was a 15-min interval between the infusion of test substances, and, when a new glucose concentration was begun, there was a 15-min wait before glucagon was initiated. The experiments with exogenous glucagon were randomized with regard to prior glucose exposure. With glucagon stimulation at a glucose concentration of 15 mg/dl, there had been prior exposure to glucose at 150 mg/dl in two experiments and to glucose at 300 mg/dl in two others. In glucagon studies with glucose at 88 mg/dl, there was prior exposure to glucose at 150 mg/dl in one experiment. In glucagon studies with glucose at 150 mg/dl, there was prior exposure to glucose at 15 mg/dl in four experiments and 88 mg/dl in one; and for the studies at a glucose concentration of 300 mg/dl, there was prior exposure to glucose at 15 mg/dl in two experiments. In six of the arginine experiments (three pancreases), there had been prior exposure to exogenous insulin. In the experiments with exogenous insulin there was prior exposure to arginine in one. In analysis of the results of these studies, there was no indication that hormone responses were influenced by prior exposure to varying glucose concentrations, exogenous insulin, or arginine. Although not rigorously proved, it appeared that pancreases were equally responsive throughout the perfusions' durations.

**Radioimmunoassays.** Efflux insulin and glucagon (antiserum 30K obtained from Dr. R. H. Unger) were measured by standard radioimmunoassay techniques.[26,27] The radioimmunoassay used for somatostatin is a modification[28] of the method of Patel and Reichlin.[29]

A specific and sensitive radioimmunoassay was used to measure pancreatic polypeptide levels in the effluent (antibody and human standard kindly provided by Dr. Ronald E. Chance, Eli Lilly Co., Indianapolis). Methods were similar to a previously published method[20] with the following modifications in assay conditions. Antibody was used at a final dilution of 1:1,000,000, and the buffer was 0.04 M borate-BSA, pH 8.0. Experimental detection limit was 5 pg per tube, and pooled normal human sera in varying dilutions were run as between-assay controls. Dextran-coated charcoal was used to separate free from bound hormone. Fifty percent displacement occurred at a dose of 41 ± 6 pg (mean ± SD).

The exogenous porcine glucagon used in these experiments contained no immunoassayable insulin, somatostatin, or pancreatic polypeptide. The exogenous porcine insulin in the perfusate did not contain measurable amounts of somatostatin or pancreatic polypeptide but did contain 10 pg/ml of glucagon immunoreactivity, and this was corrected for by subtraction.

**Data presentation.** The integrated hormone output displayed in Table 1 represents the mean output per minute of the 5-min baseline period and the 10-min glucagon-stimulated period. The means were derived from the sum of the output values for each minute. The two-tail Student's *t* test was used to determine significance. All data are expressed as mean ± standard error of the mean (SEM). When comparisons of hormone output between different perfusate glucose concentrations were made, the calculations were based on the number of perfusions (separate pancreases). For example, if there were four experiments within a given perfusion at a perfusate glucose concentration of 15 mg/dl, the mean of those four values was determined and considered as N = 1. When a rise from baseline in response to exogenous glucagon was analyzed, a paired Student's *t* test was employed using experiments rather than pancreases. This was done because only two pancreases were used for the studies done at perfusate glucose concentrations of 88 and 300 ng/dl.

The timing of the first detectable hormone responses to exogenous glucagon or arginine was done making certain assumptions. With assay error and secretory variability taken into account, it was assumed that the coefficient of variation for the mean of the baseline concentrations was 12.5%. Thus, 25% would approximate two standard deviations and 95% confidence limits. Therefore the first point that exceeded 125% of the mean of the baseline following initiation of the glucagon stimulus was considered to be the first detectable response.

## RESULTS

**Effects of exogenous glucagon.** Glucagon infused at a concentration of 100 ng/ml produced clear stimulation of somatostatin secretion at all four perfusate glucose concentrations (Figure 1 and Table 1). The peak of the first phase occurred within the second minute after initiation of the glucagon, and the second phase of increased secretion persisted throughout the duration of the glucagon infusion. There was no detectable response of pancreatic polypeptide to this concentration of exogenous glucagon. Insulin secretion was also stimulated by exogenous glucagon in a biphasic pattern, with the first phase peak coinciding with that of somatostatin (Figure 2).

The effect of perfusate glucose concentration on insulin secretion was definite (Figure 2 and Table 1). Statistical significance was determined using the unpaired two-tail Student's *t* test and the more rigorous approach of using perfusions rather than experiments (see METHODS). Baseline secretion at a glucose concentration of 300 mg/dl was greater than at 15 mg/dl ($P < 0.01$), but at 88 and 150 mg/dl it was not significantly greater than at 15 mg/dl. Glucagon-stimulated secretion at 88 and 300 mg/dl was greater than at 15 mg/dl ($P < 0.01$), whereas at 150 mg/dl the increase over 15 mg/dl was significant at $P < 0.05$. There was no significant difference in glucagon-stimulated

secretion between 88 and 150 mg/dl of glucose. In contrast, somatostatin secretion, either baseline or glucagon stimulated, was not significantly enhanced by glucose. Perfusate glucose likewise had no effect on pancreatic polypeptide secretion in these experiments. Previously published data of ours and others show a stimulation of somatostatin secretion occurring shortly after perfusate glucose is increased.[7,24,30] Pancreatic polypeptide concentrations were measured on the same samples (data unpublished), and no change of secretion was found as perfusate glucose was altered acutely.

The timing of the rapidity of response of somatostatin and insulin to exogenous glucagon was evaluated. Nineteen experiments were performed in five separate perfusions, and in 12 cases the insulin and somatostatin responses occurred in the same collection interval. In five cases insulin secretion preceded somatostatin, whereas in two the reverse occurred. Most of the initial responses were detectable in either the 30–45 s or the 45–60 s interval. The perfusate glucose concentration had no obvious influence on the response rate of either hormone. Because of the large volume of the canine pancreas the precise timing of the responses is not possible, but on the basis of dye studies (see METHODS) it can be estimated that measurable B and D cell secretion usually occurred 15–45 s after exposure of these cells to exogenous glucagon.

**Effects of exogenous arginine.** The infusion of 10 mM arginine with a perfusate glucose concentration of 15 mg/dl led to increased secretion of glucagon, somatostatin, and pancreatic polypeptide, which occurred in a biphasic pattern (Figure 3). The peak response for all three peptides was seen during the second minute after the arginine infusion was initiated. The increases of glucagon and pancreatic polypeptide, as compared with baseline values, were statistically significant ($P < 0.01$, paired $t$ test, two tails) at all points corresponding to minutes 2 through 10. The somatostatin increases at these points were also significant at $P < 0.01$ except for minutes 3, 5, and 10, which were significant at $P < 0.05$. There was no significant increase of insulin secretion, presumably because of the low perfusate glucose concentration.

In eight experiments (four perfusions), samples were

TABLE 1
Integrated hormone output from the perfused canine pancreas*

| Perfusate glucose (mg/dl) | Insulin (mU/min) | Somatostatin (ng/min) | Pancreatic polypeptide (ng/min) |
|---|---|---|---|
| | Baseline (5 min) | | |
| 15 | 3.35 ± 1.23 | 1.57 ± 0.32 | 3.21 ± 0.23 |
| 88 | 7.86 ± 4.07 | 1.40 ± 0.21 | 3.83 ± 0.59 |
| 150 | 11.39 ± 5.12 | 0.95 ± 0.15 | 3.42 ± 0.61 |
| 300 | 27.66 ± 5.05 | 1.37 ± 0.17 | 2.52 ± 0.27 |
| | Glucagon-stimulated (10 min) | | |
| 15 | 8.76 ± 2.25† | 2.79 ± 0.59† | 2.94 ± 0.21 |
| 88 | 81.96 ± 28.61† | 5.58 ± 1.06† | 4.26 ± 0.82 |
| 150 | 103.78 ± 28.93† | 3.26 ± 0.56† | 3.61 ± 0.75 |
| 300 | 217.49 ± 40.03† | 5.87 ± 0.96† | 2.76 ± 0.21 |

* Data calculated on basis of experiments.
† Significant increase from baseline at same perfusate glucose by paired Student's $t$ test, calculated on basis of experiments, $P < 0.01$.

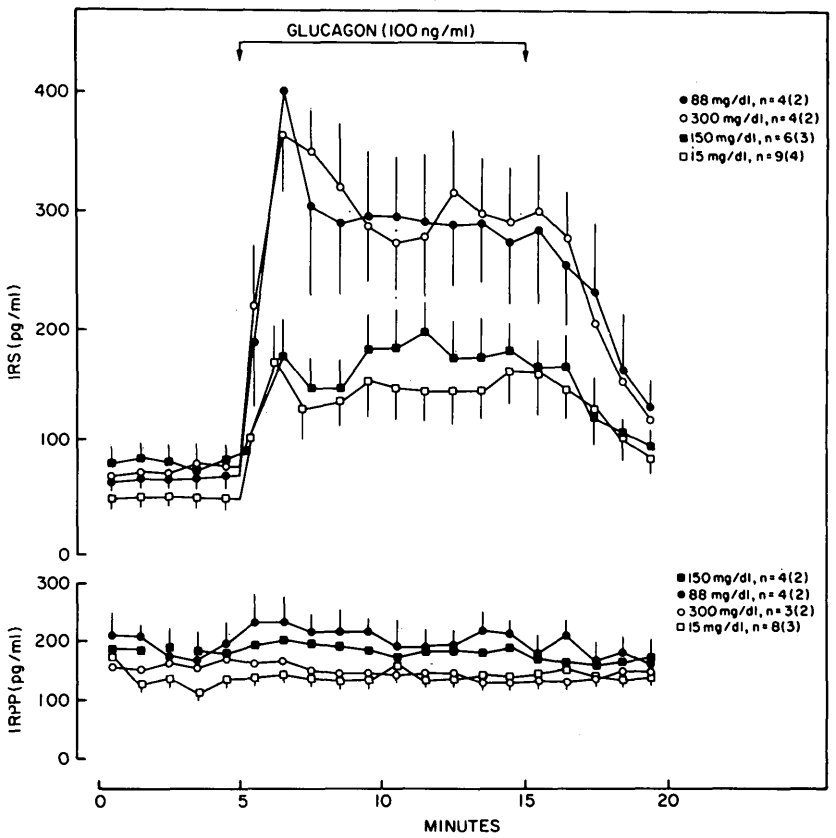



**FIGURE 1. Effect of exogenous glucagon on somatostatin and pancreatic polypeptide secretion at varying concentrations of perfusate glucose. N refers to the number of experiments, and the number in parentheses refers to the number of perfusions (individual pancreases).**

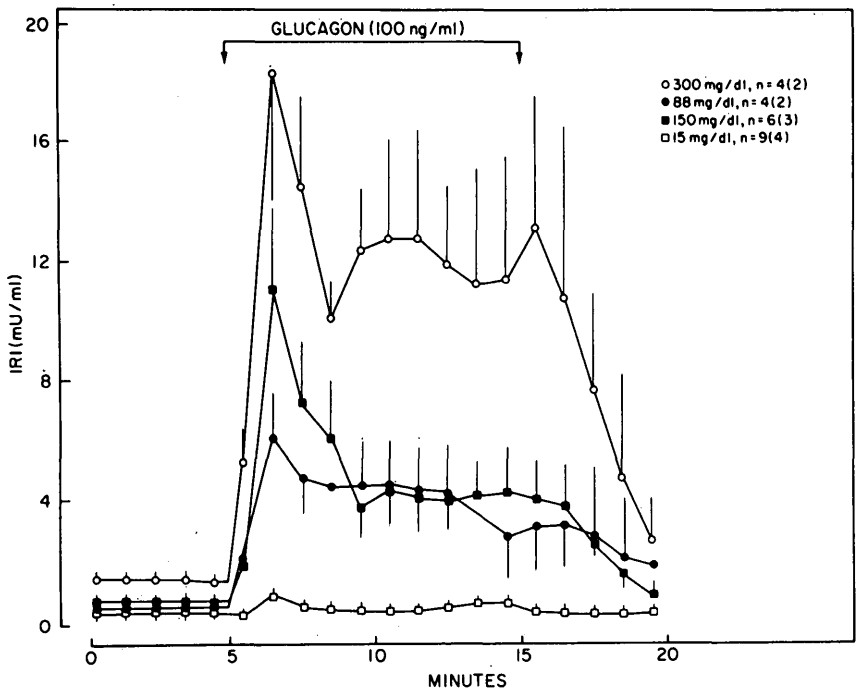
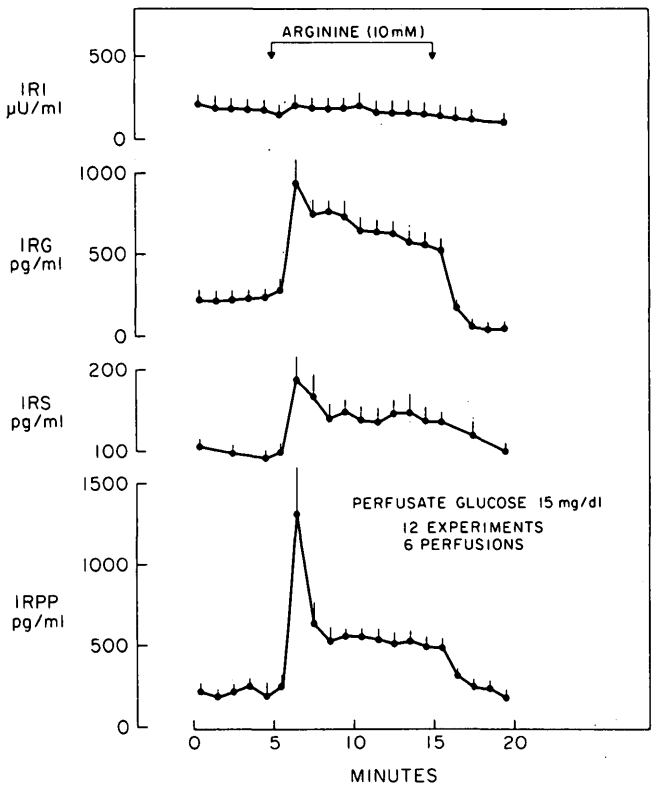



**FIGURE 2. Effect of exogenous glucagon on insulin secretion at varying concentrations of perfusate glucose. N refers to the number of experiments, and the number in parentheses refers to the number of perfusions (individual pancreases).**

taken at 15-s intervals for 3 min. The glucagon increases were first seen between 15 and 45 s in all eight, as were the somatostatin and pancreatic polypeptide responses in seven. In one experiment the rise of the latter two peptides occurred between 45 and 60 s. Thus it can be estimated that measurable A, D, and PP cell secretion usually occurred less than 30 s after exposure of the cells to arginine.

**Effects of exogenous insulin.** When exogenous porcine insulin was infused at a concentration of 50 mU/ml, with a perfusate glucose concentration of 15 mg/dl, there was no change in the secretory rate of either somatostatin or pancreatic polypeptide (Figure 4). The decline of glucagon secretion from baseline was not statistically significant. In three experiments (three separate perfusions), however, five baseline points were compared with the eight points of minutes 3 through 10, and the glucagon suppression was significant at $P < 0.001$.

**FIGURE 3. Effect of arginine on insulin, glucagon, somatostatin, and pancreatic polypeptide secretion.**




## DISCUSSION

These results indicate that exogenous glucagon stimulates somatostatin and insulin secretion in a biphasic pattern and that arginine has a similar effect on glucagon, somatostatin, and pancreatic polypeptide. The failure of arginine to elicit an insulin response can probably be attributed to the low glucose concentration of the perfusate, because, in other studies employing higher perfusate glucose, biphasic insulin release has been found.[31,32] Thus the biphasic pattern of release appears to be a general phenomenon of islet hormone secretion. The responsiveness of insulin to exogenous glucagon[33] and of insulin and glucagon to arginine[34,35] is well known. Patton et al., also using the perfused canine pancreas, described stimulation of somatostatin by exogenous glucagon,[11] but the response occurred more slowly and a biphasic pattern was not seen. The stimulation of somatostatin secretion by arginine was very similar to the earlier results reported by Patton et al.[12] The response of pancreatic polypeptide to arginine in the dog contrasts with the failure to see increased plasma pancreatic polypeptide concentrations in man during intravenous infusions of arginine.[18] Perhaps a modest response does occur in man but is obscured by hepatic uptake of the peptide.[36] Alternatively, arginine may not be as potent a secretagogue in man as it is in dogs.

Exogenous insulin has been previously shown to inhibit glucagon secretion,[37,32] and it is unclear why the effect was

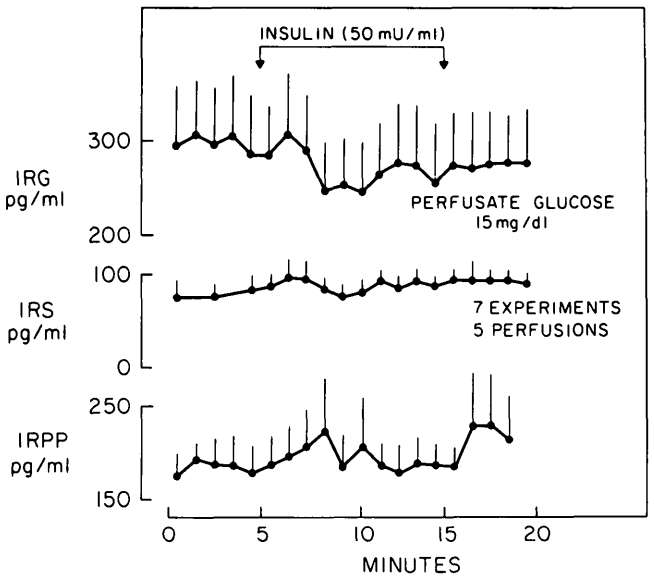



**FIGURE 4. Effect of exogenous insulin on glucagon, somatostatin, and pancreatic polypeptide secretion.**

found in the present study in only three of seven experiments. In contrast with our earlier study,[37] these infusions were done during the first 45 min of the perfusion, and perhaps there was insufficient equilibration time. Exogenous insulin had no effect on either somatostatin or pancreatic polypeptide secretion. The failure to see an acute effect of exogenous insulin on somatostatin secretion contrasts with chronic studies in rats and mice that suggest that hypoinsulinemia is associated with increased pancreatic and gastric somatostatin content, whereas hyperinsulinemia is associated with reduced content.[38–41]

It has previously been demonstrated that glucagon-stimulated insulin secretion can be markedly enhanced by an increased glucose concentration.[42] Somatostatin secretion, like insulin secretion, can be stimulated by glucose,[7,15,24,30] but the effect is comparatively modest. The failure to see a significant effect in the present experiments may be related to the experimental design employed, a minor effect being obscured by the variation of somatostatin secretion between different pancreases. The relative lack of glucose control over the D cell is further emphasized by the finding that somatostatin secretion was stimulated by arginine when the perfusate glucose concentration was low, whereas insulin secretion was not.

The demonstration that certain islet cells can be influenced by the secretory products of other islet cells raises the possibility that complex paracrine mechanisms may be operative within islets.[32,37] Such paracrine effects that may occur acutely include glucagon from the A cell stimulating the B and D cell; insulin from the B cell suppressing the A cell; and somatostatin from the D cell inhibiting the A, B, and PP cells. Our preliminary studies and a report by others[43] suggest that pancreatic polypeptide has no acute effect on the A, B, and D cells. Presently, sufficient data are not available to permit speculation about possible chronic paracrine effects.

Acute paracrine effects may occur very rapidly, as our data indicate that the B and D cells can respond to exogenous glucagon in less than 45 s, and it is likely that responses may sometimes occur in less than 15 s. It is possible that arginine-stimulated insulin and somatostatin release is initiated by the paracrine secretion of glucagon, and we are unable to convincingly rule out this possibility. Because of its large size the canine pancreas is not an ideal system for accurate timing of hormone output, and it would be expected that the perfused rat pancreas would yield more precise information.[44] Nonetheless, it is clear that these four islet hormones are released very rapidly in response to arginine, although their precise interrelationships and involvement in nutrient metabolism remain to be elucidated.

## ACKNOWLEDGMENTS

The authors thank John A. Day, Jr., Rosemary Ramseur, Janis Schwarz, Clare Mathe, and Nelleke Laurens for their skilled and conscientious technical assistance.

This work was supported by grants AM–19343, AM–20349, and AM–15019 from the National Institutes of Health and by grants from the Medical Research Service of the Veterans Administration and the Canadian Medical Research Council.

Dr. Weir is a recipient of a Research Career Development Award AM–00255 from the National Institutes of Health.

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