# Effect of Glucocorticoid and Growth Hormone Treatment on Proinsulin Levels in Humans

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**Treatment with glucocorticoids is associated with a disproportionate elevation in the PI/IRI ratio. To determine whether growth hormone—another agent capable of producing insulin resistance and changing B-cell function—also alters the PI/IRI ratio and whether growth hormone and glucocorticoids have a synergistic effect on PI and IRI levels, we examined these variables in four groups of young healthy subjects ($n$ = 8/group) after 7 days of treatment with placebo, prednisone (0.8 mg · kg $^{-1}$ · day $^{-1}$), rhGH (0.1 mg · kg $^{-1}$ · day $^{-1}$), and the combination of prednisone and rhGH. Fasting plasma glucose levels increased significantly above those of the control group in subjects receiving prednisone or prednisone and rhGH but not in subjects receiving rhGH alone. The basal concentration of IRI increased in response to prednisone, rhGH, and the combination of prednisone and rhGH. However, this increase in IRI was largely due to an increase in PI, so that the PI/IRI ratio increased from 14.7 ± 2.4% in control subjects to 33.9 ± 5.3% in subjects on prednisone ($P < 0.005$), 40.9 ± 4.3% in individuals receiving rhGH ($P < 0.001$ vs. control subjects), and 58.1 ± 9.2% in subjects receiving both prednisone and rhGH ($P < 0.001$ vs. control subjects). We suggest that this change in PI/IRI with glucocorticoid and growth hormone treatment may be due to an alteration in B-cell synthesis or release of PI. This change in the PI/IRI ratio is not dependent on fasting hyperglycemia but may contribute to the hyperglycemia often observed with these agents. Furthermore, these data show that IRI is not a reliable indicator of true insulin levels or insulin sensitivity in either growth hormone- or glucocorticoid-treated subjects. *Diabetes* 42:1082–85, 1993**

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PI/IRI, proinsulin to immunoreactive insulin ratio; PI, proinsulin; IRI, immunoreactive insulin; hGH, human growth hormone; rhGH, recombinant DNA human growth hormone; IDDM, insulin-dependent diabetes mellitus; NIDDM, non-insulin-dependent diabetes mellitus; CV, coefficient of variation; RIA, radioimmunoassay; STZ, streptozocin; ANOVA, analysis of variance.

NIDDM is associated with a disproportionate increase in the PI component of IRI (1–8). Such an alteration also has been observed during glucocorticoid administration (4,9), another situation in which islet function is diminished (10,11) and insulin resistance is present (12,13).

Growth hormone is another agent capable of inducing insulin resistance (14,15). However, in contrast to glucocorticoids, studies of growth hormone's effects in vitro suggest that it does not inhibit B-cell function but rather appears to directly stimulate insulin secretion (16,17). Nevertheless, an excess of growth hormone may, at times, be associated with the development of hyperglycemia. Thus, based on the hypothesis that growth hormone's action on the B-cell may include a change in PI release, we measured basal PI and IRI levels in normal humans who had participated in a study to determine the effects of prednisone, hGH, or a combination of the two agents on metabolism (18,19).

## RESEARCH DESIGN AND METHODS

The samples used in this study were obtained from subjects who participated in a study to evaluate the effects of prednisone and growth hormone on metabolism (18,19). The 32 apparently healthy subjects ranged in age from 18–36 yr and were within 6% of ideal body weight. All subjects had a normal 2-h postprandial glucose level after a meal containing at least 100 g of carbohydrate. None of the participants had a first-degree relative with diabetes mellitus. The study was approved

TABLE 1
Effect of prednisone, rhGH, and the combination of prednisone and rhGH administration on fasting plasma glucose, IRI, PI, and true insulin levels in normal humans

| | Control | Prednisone | rhGH | Prednisone plus rhGH |
|---|---|---|---|---|
| Glucose (mM) | 4.3 ± 0.2 | 5.9 ± 0.2* | 4.4 ± 0.2† | 6.7 ± 0.5*‡ |
| IRI (pM) | 22 ± 4 | 99 ± 8* | 37 ± 6† | 274 ± 69*‡§ |
| PI (pM) | 2.8 ± 0.3 | 32 ± 4* | 14 ± 2*§ | 190 ± 72*‡‖ |
| True insulin (pM) | 20 ± 3 | 67 ± 8¶ | 23 ± 4§ | 84 ± 14¶** |
| PI/IRI (%) | 14.7 ± 2.4 | 33.9 ± 5.3¶ | 40.9 ± 4.3* | 58.1 ± 9.2*†† |

Data are presented as means ± SE.
¶$P < 0.005$ and *$P < 0.001$ vs. controls; ††$P < 0.05$, ‖$P < 0.01$, §$P < 0.005$ and †$P < 0.001$ vs. prednisone; **$P < 0.005$ and ‡$P < 0.001$ vs. growth hormone.

by both the Institutional Review Board and Clinical Research Center Advisory Committee of the Mayo Clinic.

By randomization, four study groups were formed with eight subjects each. The control group received placebo lactose tablets and saline injections. The glucocorticoid group received 7 days of saline injections and prednisone administered at a dose of 0.8 mg · $kg^{-1}$ · $day^{-1}$ in three divided doses before breakfast, lunch, and dinner. The growth hormone-treated group received placebo tablets as well as rhGH 0.1 mg · $kg^{-1}$ · $day^{-1}$ given subcutaneously at dinner time. The fourth group received prednisone and rhGH as described above. During the study, subjects consumed a constant composition diet containing ~53% carbohydrate, 29% fat, and 18% protein.

Arterialized basal plasma samples for measurement of PI and IRI (see below) were obtained after a 22-h fast.

**Assays and calculations.** Plasma glucose was measured using the glucose oxidase method (Beckman Instruments, Palo Alto, CA). Plasma IRI was measured using a modification (20) of the double-antibody method of Morgan and Lazarow (21). This assay has an intra- and interassay CV of 8 and 12%, respectively. In this assay, the insulin antibody reacts with PI and its conversion intermediates equally to insulin on a molar basis but does not recognize insulinlike growth factor I. Thus, IRI is used to refer to total measured insulinlike molecules, which include insulin, PI, and PI conversion intermediates.

Fasting serum PI and PI intermediates were measured as previously described (22). Briefly, PI-like molecules are immunoprecipitated with a C-peptide antibody, followed by RIA of the resuspended precipitate with an insulin antibody. Although <1% of insulin is extracted during the immunoprecipitation procedure, the specific amount is quantified in each assay and corrected for in the PI calculation. Because both intact PI and PI conversion intermediates (split PIs) cross-react 100% with the insulin antibody, they are measured in the PI assay. Therefore, PI is used to refer to all PI-like molecules. In this assay, which has been further modified by antibody dilution to increase sensitivity, PI levels of 2 pM can be differentiated from 0 in 93% of cases. Values below this limit are assigned a value of 2. The assay has intra- and interassay CVs of 10 and 14%, respectively. "True insulin" is the fraction of IRI not comprised of PI-like molecules; it also is calculated when solving simultaneous equations for the PI concentration. All samples were assayed in duplicate in the same assay, thus avoiding interassay variability.

The proportion of PI that comprised IRI (PI/IRI) was calculated as PI/IRI × 100 (%).

**Statistical analysis.** Statistical analysis was performed using Statview SE + Graphics (Abacus Concepts, Berkeley, CA). Results are expressed as means ± SE. For evaluation of the effect of treatment, one-way ANOVA was performed with the Mann-Whitney *U* test used to determine statistical difference among the groups. Correction for multiple comparisons was not performed based on the argument presented by Rothman (23). $P < 0.05$ was considered significant.

## RESULTS

The effect of treatment with these different agents on fasting plasma glucose, IRI, PI, and true insulin concentrations are listed in Table 1. After 1 wk of rhGH treatment, the fasting plasma glucose concentration was similar to that observed with no therapy. In contrast, 1 wk of prednisone administration resulted in a significant increase in fasting glucose, and this was further increased when the combination of rhGH and prednisone was given. In response to administration of prednisone and rhGH either alone or in combination, both fasting PI and IRI levels increased, although the increase in IRI with rhGH administration only approached significance ($P < 0.056$). True insulin levels did not change with rhGH treatment but increased significantly during administration of prednisone, with or without rhGH.

The results of the changes in PI and IRI observed during hormone administration on the PI/IRI ratio are shown in Fig. 1. Prednisone administration resulted in a doubling of the PI/IRI ratio to 33.9 ± 5.3% compared to 14.7 ± 2.4% measured in control subjects ($P < 0.005$). Although the increase in the IRI concentration was less when the subjects were treated with rhGH, the increase was almost entirely due to PI so that the PI/IRI ratio increased to 40.9 ± 4.3% after 1 wk of rhGH treatment ($P < 0.001$ vs. control subjects). The combined administration of prednisone and rhGH further increased the proportion of IRI comprised of PI, measured as a PI/IRI ratio of 58.1 ± 9.2% ($P < 0.001$ vs. control subjects). The increased PI/IRI ratio during combination therapy was

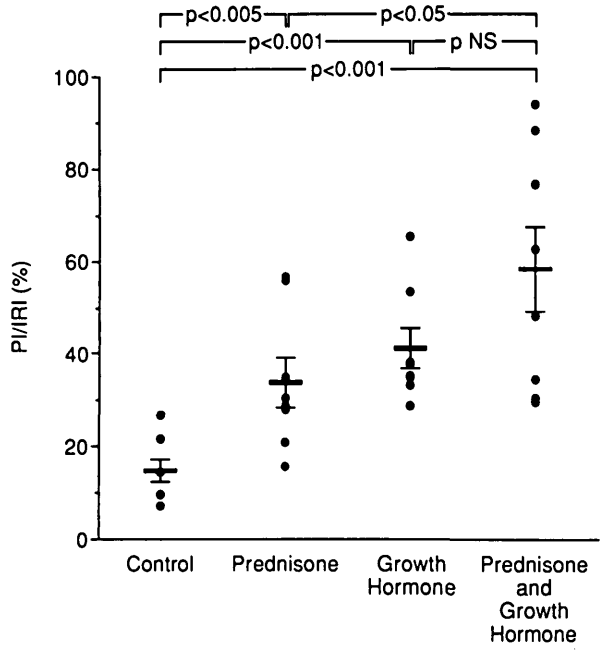


**FIG. 1. Effect of prednisone, growth hormone, and the combination of prednisone and growth hormone on the PI/IRI ratio in normal humans ($n$ = 8/group). Means ± SE are illustrated for each condition.**

significantly greater than that measured during prednisone alone ($P < 0.05$) but was not significantly increased above that observed during rhGH administration.

## DISCUSSION

The results of this study agree with the previous findings of Kitabchi et al. (9) and Ward et al. (4) who observed an increase in PI levels in normal subjects treated with glucocorticoids. In addition, this study demonstrates that when hGH is given to normal human subjects only PI increases, leading to a disproportionate increase in fasting PI levels. Combination therapy with glucocorticoids and growth hormone results in a marked elevation in plasma PI concentrations and this increase is again disproportionate so that approximately 58% of the basal IRI is comprised of PI.

The finding of a disproportionate increase in PI levels during growth hormone treatment adds another dimension to the in vitro observation that growth hormone is capable of directly altering B-cell function (16,17). In vitro islet studies have suggested that hGH is capable of directly stimulating IRI release, but only after a period of time in which synthesis of new insulin has occurred. Thus, it seems feasible that the alteration in insulin production and release by the B-cell also may include a change in the proportion of IRI comprised of PI, which is manifested as an increase in the basal PI/IRI ratio. Although we have not accounted for differences in peptide clearance that could be responsible for the alteration in PI/IRI, it does not seem likely that such changes in clearance explain the large differences in the PI/IRI ratio observed in this study. This belief is based on the fact that insulin receptor function is reduced in acromegalics (24), and, if this occurred in our subjects, insulin clearance should be unchanged or reduced. Such a reduction in insulin clearance should increase IRI levels and, thus, the PI/IRI ratio should decrease. An alternative explanation for our observation would be that renal clearance of PI and its conversion intermediates is reduced by growth hormone, whereas IRI clearance is not, thus increasing the PI/IRI ratio. We have not systematically excluded this possibility and are unaware whether anyone has examined this issue. The observation that alterations occur in PI levels during growth hormone administration is similar to that previously documented with glucocorticoids.

The observation that a disproportionate increase in PI levels occurs during growth hormone administration without concurrent hyperglycemia may be pertinent to the disproportionate proinsulinemia in NIDDM (1–8). In a study conducted in Pima Indians, a linear relationship between the fasting plasma glucose concentration and the degree of elevation in PI/IRI was demonstrated (8). However, no firm conclusion could be reached whether this relationship was the result of a primary B-cell abnormality, the degree of which was manifested in part by the magnitude of increase in PI/IRI, or whether this represented an effect of glucose to alter B-cell function by a "toxic" effect. Data from this study demonstrating an increase in PI/IRI without hyperglycemia support the explanation that the altered PI/IRI in NIDDM may be a manifestation of an underlying islet abnormality. A similar B-cell abnormality was produced in baboons that were given a dose of STZ sufficient to produce a mild impairment of B-cell function without fasting hyperglycemia (25). The abnormality also has been observed in individuals at high risk of developing IDDM (26).

Finally, the data herein reiterate the complexity of interpreting basal IRI levels. In conditions associated with insulin resistance and increased B-cell secretory demand, basal IRI levels are elevated and often are used as an index of the degree of insulin resistance. Because most insulin antibodies do not discriminate between insulin and other insulinlike molecules, such as PI and its conversion intermediates, one must be cautious because simply measuring IRI may not necessarily provide a proportional reflection of the change in circulating true insulin. This is especially true in NIDDM or when an individual is receiving medications such as glucocorticoids or growth hormone, which are known to alter the proportion of circulating PI. Under such conditions, IRI will consist of varying proportions of PI and its conversion intermediates, as well as true insulin. Thus, simply estimating insulin sensitivity based on the fasting IRI concentration may not be reliable. In such cases, direct quantification of insulin sensitivity may be necessary.

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