Cortisol increases plasma insulin-like growth factor binding protein-1 in humans

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Insulin-like growth factor binding protein-1 (IGFBP-1) modulates the metabolic and mitogenic actions of the IGF peptides. Previous studies have established insulin as the major regulator of plasma IGFBP-1 in humans, acting to suppress hepatic IGFBP-1 synthesis. In this study, we investigated the regulation of plasma IGFBP-1 by cortisol in humans, independent of insulin. Following an overnight fast, six healthy adult volunteers received a euglycemic pancreatic clamp (somatostatin, 0.12 µg·kg⁻¹·min⁻¹; GH, 3 ng·kg⁻¹·min⁻¹; insulin, 0.05 mU·kg⁻¹·min⁻¹) to block endogenous insulin secretion and to control glucose and plasma hormone concentrations at desired levels. Three hours after the initiation of the pancreatic clamp, each subject received an additional 360 min infusion of either cortisol (2 µg·kg⁻¹·min⁻¹) or saline on separate occasions and in random order. Plasma cortisol concentrations increased from 220 to 970 pmol/l during the cortisol infusion. Insulin concentrations were maintained at approximately 30 pmol/l throughout saline and cortisol infusions. Plasma IGFBP-1 concentrations increased threefold in response to hypoinsulinemia, reaching plateau values of ~140 µg/l with saline infusion. During cortisol infusion, IGFBP-1 levels increased to ~300 µg/l. Over the 360 min study period, the integrated response of plasma IGFBP-1 to cortisol infusion was 314% greater than to saline infusion (p < 0.01). Our data confirm that, under conditions of hypoinsulinemia, cortisol is a significant modulator of plasma IGFBP-1 in humans.

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The insulin-like growth factors (IGFs) are important regulators of cell metabolism, differentiation, and proliferation (1, 2). Since IGFs are secreted as they are produced, orderly delivery to the target tissues and ultimate bioactivity of these growth-promoting peptides depends, to a large extent, on a family of specific, high affinity, diversely functioning IGF binding proteins (IGFBPs [1, 2]). The first of these proteins to be characterized, IGFBP-1, is produced primarily by liver, ovarian granulosa cells, and secretory or decidualized endometrium (3–5). Although the physiological role of IGFBP-1 has not yet been clearly defined, there is strong evidence from both in vivo and in vitro studies that IGFBP-1 is a potent inhibitor of IGF action (6–9).

Numerous clinical investigations have indicated that insulin plays a major role in the regulation of IGFBP-1. Plasma levels of IGFBP-1 are elevated in patients with poorly controlled insulin-dependent diabetes mellitus (IDDM) and during fasting, and rapidly decline after a meal or with insulin treatment (10–13). We have demonstrated that peripheral insulin concentrations of 70–90 pmol/l are sufficient to achieve maximal suppression of IGFBP-1 production in vivo (10, 14). Furthermore, in vitro studies show that insulin can directly inhibit hepatic IGFBP-1 expression (15–18).

Animal studies indicate that glucocorticoids may also play a role in IGFBP-1 regulation. Dexamethasone dramatically increases IGFBP-1 mRNA abundance in rat hepatoma cells (17–19). However, the effect of insulin dominates, both preventing and reversing dexamethasone-induced increases in IGFBP-1 gene transcription (17, 18). In vivo rat studies are consistent with dual regulation of IGFBP-1 by insulin and glucocorticoids (20–22). A role for glucocorticoids in regulating IGFBP-1 in humans has not been established, since available in vivo studies have not controlled for insulin. In normal subjects, dexamethasone treatment resulted in compensatory hyperinsulinemia and a decrease in plasma IGFBP-1 levels (23). In another study, the normal early morning rise in plasma IGFBP-1 associated with overnight fasting was absent in patients with Cushing’s syndrome (24), but this, again, may represent a secondary effect of glucocorticoids mediated by increased insulin levels. Therefore, we investigated the effect of cortisol on plasma IGFBP-1 during controlled hypoinsulinemia to test the hypothesis that glucocorticoids have
an independent effect to increase plasma IGFBP-1 concentrations in humans.

Subjects and methods

Subjects and protocol

Investigations were approved by the Mayo Clinic Institutional Review Board. Six healthy, non-obese volunteers (3M, 3F; age 22 ± 1 years) were studied concurrent with a separate investigation of in vivo regulation of lipolysis (25). Details basic to understanding the structure and assessing the validity of the experimental design are repeated here for clarity and convenience. Women were studied during the follicular phase of their menstrual cycle, which avoids the variable of secretory endometrium-derived IGFBP-1.

Each subject was admitted to the Mayo Clinic General Clinical Research Center the evening before the study and given a standard meal. After an overnight fast, baseline blood samples were collected and an 18-gauge infusion catheter was placed in a forearm vein: a separate 18-gauge catheter was inserted for sampling of arterialized venous blood (25).

A pancreatic clamp consisting of infusions of 0.12 µg·kg⁻¹·min⁻¹ somatostatin, 3 ng·kg⁻¹·min⁻¹ growth hormone, and 0.05 mU·kg⁻¹·min⁻¹ insulin was initiated at 07.00 (t = -180 min) and continued for the full 9 h study period. At 10.00 (t = 0), an infusion of saline or 2 µg·kg⁻¹·min⁻¹ cortisol was started and continued for 360 min. Each subject was studied on two occasions, with saline or cortisol administered in random order. Fifty percent dextrose was infused throughout the study to maintain plasma glucose concentrations between 4.7 and 5.6 mmol/l.

Blood was sampled at 15-min intervals 45 min prior to the start of the experimental infusions, at 30-min intervals for the first 60 min after initiation either of cortisol or saline, and hourly thereafter until the conclusion of the study. Plasma glucose concentrations were monitored at 15-min intervals.

Materials

Recombinant DNA-derived regular human insulin (Humulin, 100 U/ml) and human GH were kindly provided by Eli Lilly and Co. (Indianapolis, IN) and Genentech, Inc. (South San Francisco, CA), respectively. Somatostatin was obtained from Bachem, Inc. (Torrance, CA).

Assays

IGFBP-1 was measured by RIA using a polyclonal rabbit antihuman IGFBP-1 antiserum, as detailed in previous publications (10, 14, 15, 26). This antiserum recognizes a single peak at ~35 kD in serum after size exclusion chromatography (26). Pure IGFBP-3 does not displace in the assay (cross-reactivity <1%). All samples were run in a single assay. The intraassay coefficient of variation at 7.86 µg/l is 13.4 ± 3.8%, with an assay detection limit of 1 µg/l.

Standard RIAs were used to measure plasma concentrations of insulin, C-peptide, cortisol, growth hormone, and glucagon (25). Plasma glucose concentrations were determined using a glucose analyzer (Yellow Springs Instruments, Yellow Springs, OH).

Statistical analyses

Data are presented as mean ± SEM. RIA data were analyzed using a 4-parameter logistic curve fit. Area-under-the-curve was calculated using the trapezoidal rule. Comparisons were made by paired t-test, with statistical significance defined as p < 0.05.

Results

Plasma hormone concentrations during infusions

After an overnight fast, plasma insulin concentrations ranged from 35 to 60 pmol/l, equivalent to portal levels of 90–140 pmol/l (10, 27). During the pancreatic clamp, which effectively equalizes peripheral and portal insulin concentrations, plasma insulin concentrations were reduced and maintained at approximately 30 pmol/l (range of 25–53 pmol/l). Corresponding C-peptide levels during the clamp were 0.01–0.02 nmol/l. As reported
previously (25), the mean baseline cortisol level was 220 nmol/l, and this level was constant during the saline infusion. Plasma cortisol increased during cortisol infusion to 600 nmol/l during the first 30 min and showed a more gradual subsequent rise to ~970 nmol/l at the end of the study. Plasma glucagon (~135 ng/l) and growth hormone (~2 μg/l) did not change during the study.

Cortisol regulation of IGFBP-1

Mean plasma IGFBP-1 concentrations are presented in Fig. 1. Prior to initiation of the pancreatic clamp, IGFBP-1 values were 42 ± 12 μg/l. Three hours later, at commencement of saline or cortisol infusions, IGFBP-1 levels had increased two- to threefold. During saline infusion, IGFBP-1 appeared to reach steady-state levels of ~140 μg/l after 120 min, or 5 h after initiation of the pancreatic clamp. During cortisol infusion, IGFBP-1 concentrations reached apparent steady-state levels of 300 μg/l after 300 min. The variability seen in Fig. 1 could be accounted for, in large part, by intersubject differences in baseline IGFBP-1 values. For example, the range in IGFBP-1 values for t = 0 (saline infusion) was 60–277 μg/l. Even so, the response to cortisol was striking, and a significant difference between saline and cortisol infusions was observed at 240, 300, and 360 min. At 240 min, five of six subjects showed an increase in IGFBP-1 levels during cortisol infusion; by 300 min, all six subjects had markedly elevated IGFBP-1. To better analyze treatment effect, the data were expressed relative to plasma IGFBP-1 values at the start of saline or cortisol infusion for each subject. The integrated response of IGFBP-1 to cortisol was 314% greater than to saline over the 360 min experimental period (p < 0.01).

Discussion

This investigation is the first to determine that cortisol independently increases plasma IGFBP-1 concentrations in humans, results which are consistent with in vivo rat studies (21, 22). Previous human studies indicated that high glucocorticoid levels were associated with reduced plasma IGFBP-1 levels (23, 24). However, those studies may have been compromised by glucocorticoid-associated hyperinsulinemia. As in vitro studies have demonstrated, the suppressive effect of insulin on hepatic IGFBP-1 synthesis can override the stimulatory effect of glucocorticoids (17, 18). An essential component of the present protocol was the elimination of an insulin effect by use of a pancreatic clamp. With this technique we were able to maintain peripheral and portal insulin at sub-fasting concentrations, and well below levels that suppress hepatic IGFBP-1 production (10, 14). Furthermore, the relatively long study period enabled us to achieve equilibrium conditions for IGFBP-1 during saline infusion and to observe the stimulatory effect of cortisol. In vitro studies also showed that significant increases in IGFBP-1 protein in response to dexamethasone required four to six hours (17, 19).

Although the mechanism underlying cortisol’s effect on plasma IGFBP-1 levels cannot be directly determined from our data, other studies have suggested that glucocorticoids act to enhance hepatic IGFBP-1 expression. Dexamethasone increases IGFBP-1 mRNA abundance in rat liver in vivo (21, 22), and in vitro studies clearly demonstrate transcriptional regulation of IGFBP-1 gene expression by dexamethasone and insulin in rat hepatoma cells (17–19). In contrast, dexamethasone had no apparent stimulatory effect on IGFBP-1 secretion by a human hepatoma cell line (HepG2) or human fetal liver explants, while insulin had potent inhibitory effects in these systems (28, 29). Although apparently discordant, these differing results may reflect the nature of the model systems rather than a species-specific response. Indeed, in the presence of dibutyryl cAMP and theophylline, dexamethasone stimulates IGFBP-1 promoter activity, mRNA expression and protein secretion in HepG2 cells (16). It is also possible that cortisol-related changes in IGFBP-1 metabolism and clearance contribute to the increases in plasma IGFBP-1 in our study.

The dominant role of insulin over glucocorticoids in regulating IGFBP-1 in humans is supported by the low IGFBP-1 levels seen in Cushings’s syndrome and during dexamethasone treatment (23, 24), conditions associated with hyperinsulinemia. Nevertheless, cortisol could be a significant modulator of IGFBP-1 in situations where insulin secretion is reduced as in prolonged fasting and starvation, absent as in IDDM, or compromised as in acute stress. The functional significance of elevated IGFBP-1 is unclear; however, IGFBP-1 inhibits IGF action in a variety of in vitro systems (6–8). Unterman and Phillips described glucocorticoid-induced serum inhibitors of IGF bioactivity, which appear to include IGFBP-1 (20, 30). Moreover, acute infusion of IGFBP-1 has recently been shown to increase glucose levels (9), raising the possibility that IGFBP-1 inhibits IGF-mediated glucose utilization. Thus, cortisol-induced increases in IGFBP-1 may contribute to the glucose intolerance seen in pathophysiological conditions of glucocorticoid excess and insulinopenia. Additional studies will be needed to clarify the interrelationship of cortisol and IGFBP-1 and its influence on carbohydrate metabolism.

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