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Pulsatile Secretion of Growth Hormone (GH) Persists During Continuous Stimulation by CJC-1295, a Long-Acting GHRH Analog

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Abstract

Context: Pulsatile GH secretion is considered important for many of the hormone's physiologic effects. Short-term GHRH infusions enhance GH pulsatility and increase IGF-1, but the short GHRH half-life limits its therapeutic use. A synthetic GHRH analog (CJC-1295) that binds permanently to endogenous albumin after injection (t1/2 = 8 d) stimulates GH and IGF-1 secretion in several animal species and in normal human subjects and enhances growth in rats.

Objective: To assess GH pulsatility after a single injection of CJC-1295 and determine which GH secretion parameters correlated to the increase in IGF-1 production.

Methods: GH pulsatility was assessed by 20-minute blood sampling during an overnight 12 hr period in healthy 20-40 year old men prior to and one week after injection of either 60 or 90 μg/kg CJC-1295.

Results: GH secretion was increased after CJC-1295 administration with preserved pulsatility. The frequency and magnitude of GH secretory pulses were unaltered. However, basal (trough) GH levels was markedly increased (7.5 fold; $p < 0.0001$) and contributed to an overall increase in GH secretion (mean GH levels: 46%; $p<0.01$) and IGF-1 levels (45%; $p<0.001$). No significant differences were observed between the responses to the two drug doses. The IGF-1 increases did not correlate with any parameters of GH secretion.

Conclusions: CJC-1295 increased trough and mean GH secretion and IGF-1 production with preserved GH pulsatility. The marked enhancement of trough GH levels by continuous GHRH stimulation implicates the importance of this effect on increasing IGF-1. Long-acting GHRH preparations may have clinical utility in patients with intact pituitary GH secretory capability.

Growth hormone (GH) is secreted from the anterior pituitary in a pulsatile manner as a result of alterations in the secretion of the hypothalamic hormones, growth hormone-releasing hormone (GHRH), which stimulates GH synthesis and release, and somatostatin, which inhibits GH release (1, 2). Ghrelin, a peptide produced primarily in the stomach, also participates in the regulation of GH secretion (3), but its role is not fully understood. In humans, up to 8-10 pulses of GH are secreted in a 24 hour period (4). Evidence for the importance of the pulsatility or intermittency of GH secretion in promoting linear growth was provided by Jansson et al (5). Many of the biological effects of GH are mediated by its stimulation of IGF-1 production. Mean GH concentration, reflected by GH peaks is correlated with serum IGF-1 concentration, but no correlation has been observed between basal GH release rate and serum IGF-1 (6).

GH is used primarily for the treatment of GH deficiency in children and adults. It has also been used in conditions with presumed functional GH deficiency, including Turner syndrome, Prader-Willi syndrome, children born small for gestational age, chronic renal insufficiency-associated growth retardation, and children with idiopathic short stature and HIV-associated lipodystrophy, severe burns, the short bowel syndrome, and the wasting syndrome associated with chronic obstructive pulmonary disease. The adverse effects of GH therapy (arthralgias, carpal tunnel syndrome, hyperglycemia, insulin resistance, glucose intolerance and fluid retention) may result in a net adverse impact, despite the beneficial effects of the therapy. The adverse effects are usually attributed to the dose of the hormone, but may also be related to the manner in which GH is administered. Exogenous GH administration, given once daily or at more prolonged intervals, does not mimic the endogenous pulsatile pattern of hormone secretion and the extremely high serum levels of GH after an injection may contribute to the diversity and degree of many of the observed adverse effects.

Growth hormone- releasing hormone (factor) (GHRH [GRF]) is a 44 amino acid peptide, with full biological activity residing in its first 29 amino acids (7). GHRH is necessary for endogenous pulsatile GH secretion and is also required to achieve optimal statural growth. Because of its effects, GHRH may represent an alternative to GH therapy in patients with an intact pituitary that would minimize the side effects associated with long term GH administration. Since the quantity of GH release induced by GHRH will be limited by the ambient IGF-1 levels, which exert a negative feedback effect, the risk of side effects associated with excessive GH secretion may be lower than with GH therapy. In addition, treatment with GHRH should result in the secretion of the whole family of GH peptides normally secreted by the pituitary, not just the 22-kDa form provided by recombinant human GH, which may have yet unrecognized beneficial effects.

Growth promoting effects and safety of GHRH administered to children with growth hormone deficiency (GHD) have been documented by several groups (8-10). Various regimens, including intravenous, pulses, intranasal delivery, subcutaneous injections and continuous infusion have been evaluated and the results show that the growth promoting effect of GHRH is correlated with the dose and frequency of administration. No unexpected adverse reactions have been seen after prolonged treatment regardless of the mode of administration (11, 12).

The half -life of GHRH after intravenous injection is 10-12 minutes (13) and represents the major limitation for its use as a therapeutic agent. In an attempt to overcome this shortcoming, a long-acting analog of GHRH has recently been developed (DAC-GRF [CJC-1295], ConjuChem Inc, Montreal Canada) that binds to endogenous serum albumin after subcutaneous administration, thereby prolonging its duration of action (14). The core therapeutic moiety of CJC-1295 is $GHRH_{1\text{-}29}NH_2$, which contains the full biologic activity of $GHRH_{1\text{-}44}NH_2$ modified

by substitution of four amino acids that renders the compound resistant to proteolytic cleavage. GRF is linked by the amino acid, lysine, to maleimidoproprionic acid, a reactive chemical that binds to unpaired thiol (sulfhydryl) groups. The predominant free thiol group available for binding after parenteral administration is the single unpaired cysteine (cysteine-34) in serum albumin. At least 90% of CJC-1295 binds covalently to albumin, with trace amounts found bound to fibrinogen and to IgG. No other chemical species have been found bound to DAC-GRF after administration after *in vivo* administration. This binding extends the half-life of the active pharmacophore to 70-90% that of serum albumin, resulting in a markedly prolonged duration of action in several animal species (15). In humans the half life of DAC-GRF has been reported to be 8-10 days (16).

Preliminary studies in humans demonstrated safety and tolerability of the drug up to an injection dose of 125 μg/kg and a significant increase in both GH and IGF-I levels for at least seven days (16). Although this study was not designed to assess the pulsatility of GH secretion, the results suggested that pulsatile secretion might be occurring. The present study was therefore designed to determine whether GH pulsatility would be maintained after an injection of CJC-1295. In addition, the study examined various parameters of GH secretion to determine which if any, were most important for the increase in IGF-1 production.

Subjects and Methods

The study was performed using a protocol approved by the University of Illinois at Chicago Institutional Review Board and all subjects gave informed, written consent. The study was conducted in the General Clinical Research Center of the University of Illinois Medical Center

Subjects and Study Drug

Twenty (20) healthy men with BMI of ≤ 25 (22.4 ±0.5; mean ±S.E.M.) and ages 20-34 (25.0 ±1.6) served as subjects. They underwent a control 12 h overnight sampling (7 PM through 7 AM) of GH and IGF-1 levels using a 20-minute sampling frequency from an indwelling forearm venous catheter) connected to tubing that permitted passage through a wall sleeve and sample collection in an adjacent room. CJC-1295 was administered at the end of the sample collection period and repeated sampling of GH and IGF-1 levels was performed one week after drug administration.

CJC-1295 was provided by ConjuChem, Inc (Montreal Canada). A 60 μg/kg dose was injected subcutaneously in four initial subjects. This dose in the previously published study resulted in an increase of GH levels of 125% and IGF-I levels of 80% (16). The desired increase in mean GH levels for the present study was 100% and in mean IGF-I levels was 75%. Power estimates indicated that eight evaluable subjects would be required to demonstrate significant differences at an 80% probability level and an $\alpha = 0.05$. The results in the first four subjects were analyzed to determine whether these target increases were achieved. If the target goals were not achieved, the dose was to be increased to 90 μg/kg and an additional eight subjects studied.

GH and IGF-1 Measurements

Serum GH and IGF-1 levels were measured by Esoterix, Inc (Calabasas, CA). Pre- and post-drug studies from individual subjects were analyzed in the same assay. GH was measured using a chemiluminescent method with an assay sensitivity of 0.05 ng/ml and intra- and inter-assay coefficients of variation of 5.8% and 9.0%. Values beneath the detectable limit of the assay were changed to 0.05 ng/ml for purposes of statistical analysis. IGF-1 was measured by a competitive

binding radioimmunoassay, with a sensitivity of 10 ng/ml and intra- and inter-assay coefficients of variation of 5.8% and 9.5%.

Statistics

The effects of the drug were determined by comparing the responses in individual subjects in the studies before and after drug administration, using a paired t-test and considering differences of <0.05 significant. GH values were subjected to log transformation to equalize variance. Sample size was determined using published literature results on the variability of the different pulsatility components as the basis for a power analysis. The likelihood of achieving a significant difference with an increase of 100% in each of the parameters (pulse number, pulse height, pulse duration, area of secretion peak, nadir level and mean level) required a population size of 8 subjects, at a power of 80% and $\alpha=0.05$. This sample size was also calculated to be sufficient to show significant differences in 3 of the parameters (pulse height, pulse duration and mean level) if the increases were only 75%. Regression analysis was performed to assess the relationships between the increase in the IGF-1 levels and various parameters of GH secretion (peak GH, mean GH, trough GH, number and mean amplitude of GH pulses), using SPSS v.13 (Chicago, IL). Pulsatility was initially assessed using Cluster software. However, a manual analysis proved to be more useful because of sampling frequency and study duration.

Results

After analyzing the results of the first four subjects injected with 60 μg/kg CJC-1295, the mean increase achieved in IGF-1 was found to be only 40%, instead of the expected 75% (16). Consequently, 8 additional subjects were studied at a dose of 90 μg/kg. A comparison of the results revealed no significant differences in any of the parameters of GH or IGF-1 secretion

between the two groups. Therefore, the two groups were combined for the analyses shown below. All of the differences shown to be significant for the combined group were also present when the analysis was restricted to only the high dose.

Pulsatility

Pulsatility was preserved in all subjects seven days after the CJC-1295 administration. There were no significant changes in the frequency of pulses or in mean or maximal peak heights after CJC-1295. The mean number of peaks at baseline was 3.5 ± 0.3 (S.E.M.) with a range of 2 to 5 and post drug administration was 3.6 ± 0.3 with a range of 2 to 6. The results were similar in both the low-dose and high-dose groups (Table 1). The maximum peak GH level was 12.2 ± 2.6 ng/ml in the control study and 11.8 ± 2.8 after the administration of the CJC-1295 and the mean peak GH values were 6.7 ± 1.4 and 6.1 ± 1.1, respectively. None of these differences exhibited statistical significance.

Serum GH

Mean GH values in the control study ranged from 0.58 to 5.17 ng/ml, with a mean of 1.79 ± 0.39 ng/ml. Mean values after CJC-1295 increased by 46% to 2.62 ± 0.61 ($p < 0.01$). Paired analysis revealed no differences in either the absolute or the percentage increases between the two doses of CJC-1295.

Trough GH values in the control study ranged from 0.05 to 0.09 ng/ml (0.058 ± 0.004). Values were at or beneath the lowest detectable assay value in 8 of the 12 subjects in the control study. Mean trough values after CJC-1295 increased 7.5 fold to 0.435 ± 0.109 ng/ml ($p<0.0001$) and were above the least detectable value in all subjects. Increases in trough values occurred in all 12 subjects (Figure 2). The overall duration of trough periods was not affected by CJC-1295. Semi-

logarithmetic plots of four representative secretory patterns that illustrate the trough changes are shown in Figure 3.

Serum IGF-1

Mean IGF-1 values in the control study were 165 ± 10 ng/ml. IGF-1 levels were increased in every subject one week after CJC-1295 injection (Figure 4). Mean IGF-1 increased to 240 ± 13 ng/ml, representing a 44% increase ($p<0.001$). IGF-1 levels did not exceed the upper limit of normal in any of the subjects. No correlations were observed between the change in IGF-1 levels and those in any of the parameters of GH secretion.

Adverse effects

No serious adverse effects were observed in any of the subjects. The most commonly observed effects were an increase in heart rate that was dose dependent, and transient redness and tenderness at the injection site that appeared to be dose independent. All adverse effects were of short duration and not considered a problem by the subjects. None of the subjects experienced any of the adverse effects commonly associated with GH therapy.

Discussion

The results of the present study have demonstrated the persistence of GH pulsatility after seven days of continuous exposure to a long-acting GHRH analog (CJC-1295). This finding was suggested by a previous study using the same analog but not designed to address the issue of GH pulsatility (16). They were also observed after shorter periods (6-24 hrs) of intravenous infusions of GHRH at higher doses ((17, 18).

In observational studies in which portal-hypophyseal blood was samples in unanesthetized sheep, GH pulsatility has been linked primarily to pulses of GHRH (19). In the present study, endogenous GHRH pulsatility was rendered inconsequential because of the nearly 100x greater levels of the GHRH analog (1-2 ng/ml) in the systemic circulation one week after injection (16), as compared to endogenous GHRH levels in the portal circulation (10-30 pg/ml) (19). Whether the persistent GH pulsatility is due to a reciprocal decrease in somatostatin concentrations or to an intrinsic pulsatility of the somatotrope cannot be assessed from the present experimental design.

No changes in pulse frequency or maximal pulse height were observed in the present study, in contrast to the enhanced pulse height seen in the study by Evans et al (18). This difference is most likely explained by the higher quantity of GHRH infused in that study, since dramatically elevated GH pulses are seen in patients with ectopic GHRH secreting tumors (20) where GHRH levels often reach the high ng/ml levels (21). It is also possible that a setting in which GH pulsatility is less pronounced, e.g., older or obese subjects and a different time window (day rather than night) might permit demonstration of enhanced pulse height.

Despite the absence of increased pulse height or frequency, mean GH secretion in the present study was increased by nearly 50% and is best explained by the increase in basal (or trough) levels of GH secretion. During the control study, GH levels were either undetectable or at the limit of detectability using a high sensitivity chemiluminescent GH assay in 8 of the 12 subjects for considerable periods during the control study. Trough levels were increased in every subject after CJC-1295 injection with a mean increase of seven fold. When trough values were defined as the mean of all values with three fold of the least detectable assay value in individual subjects, similar increases were observed (data not shown).

These results support the concept that an important role of GHRH is to raise the intrinsic secretory level of the somatotrope. It has been argued that somatostatin tone is the critical hypothalamic hormone affecting intrinsic (or "basal") GH secretion and although this cannot be excluded from the present results, one would expect endogenous hypothalamic somatostatin secretion to be increased, rather than decreased, during constant exposure to GHRH that results in elevated GH and IGF-1 levels.

Attempts to correlate changes in the various parameters of GH secretion with those in IGF-1 levels in individual subjects were unsuccessful. This may have been due to the small number of subjects and/or the fact that the study examined only a single time point (7 days) after the onset of exposure to the GHRH analog. Nevertheless, the overall results strongly suggest the importance of increases in basal (trough) GH secretion as a contributor to the increase in IGF-1 secretion. Minimal elevations in basal GH levels are also associated with significant increases in IGF-1 levels in a small subset of patients with acromegaly (22).

The mean increase in IGF-1 levels of just under 50% was less than had been observed in the initial studies with CJC-1295 (16). This is best explained by the younger age of the subjects and their lower lean body mass, both of which likely contributed to their more robust GH secretion during the control study. There may have also been a more active GH-IGF-1 feedback control system, although the effects of age and lean body mass on GH feedback are not well understood.

It is noteworthy that despite the nearly 50% increase in GH secretion and IGF-1 levels, some of which reached the upper limit of normal, none of the subjects experienced any adverse effects attributable to excessive GH or IGF-1. This may have been due to the short duration of the study,

but may also reflect a protective feedback mechanism to limit the extent of CJC-1295 stimulation of GH secretion. Based on the latter assumption, the use of CJC-1295 or other GHRH analogs may confer an intrinsic advantage over that of GH as a therapeutic agent in those patients with an intact pituitary GH secretory capacity.

Acknowledgements

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Legends to Figures

Figure 1

Patterns of GH secretion during a 12-hour sampling period (7 PM to 7 AM) before (open circles) and 1-week after (closed circles) an injection of CJC-1295, 90 μg/kg, in four subjects.

Figure 2

Trough (lowest) serum GH values during an overnight 12-hour sampling period before (open bars) and 1-week after (closed bars) an injection of CJC-1295. Shown on the left are the results in individual subjects and, on the right, the means ± S.E.M.

Figure 3

Semi-logarithmic plot of the patterns of GH secretion during a 12-hour sampling period (7 PM to 7 AM) before (open circles) and 1-week after (closed circles) an injection of CJC-1295, 90 μg/kg, in four subjects.

Figure 4

Serum IGF-1 values during an overnight 12-hour sampling period before (open bars) and 1-week after (closed bars) an injection of CJC-1295. Shown on the left are the means of IGF-1 values in individual subjects from the first three samples of the overnight 12-hour sampling period. Shown on the right are the group means ± S.E.M.

Figure 1

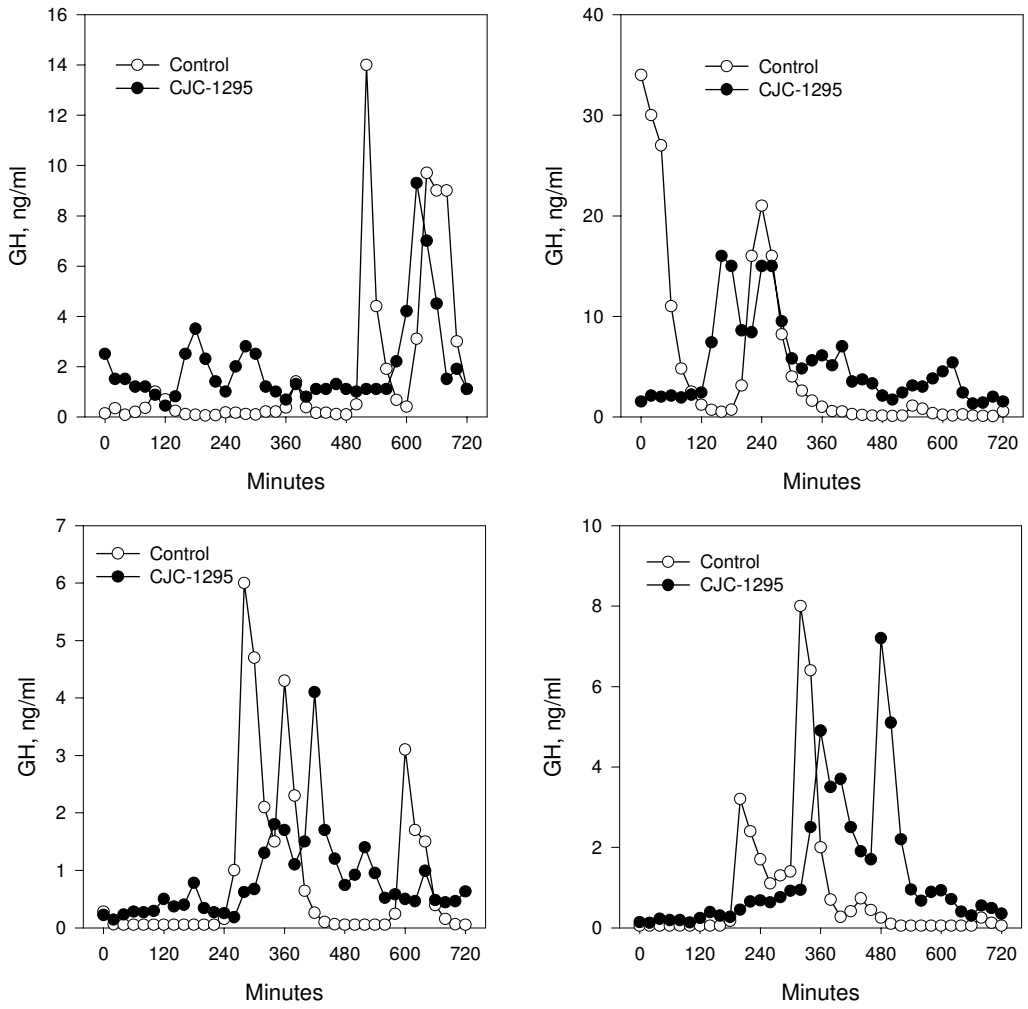


Figure 2

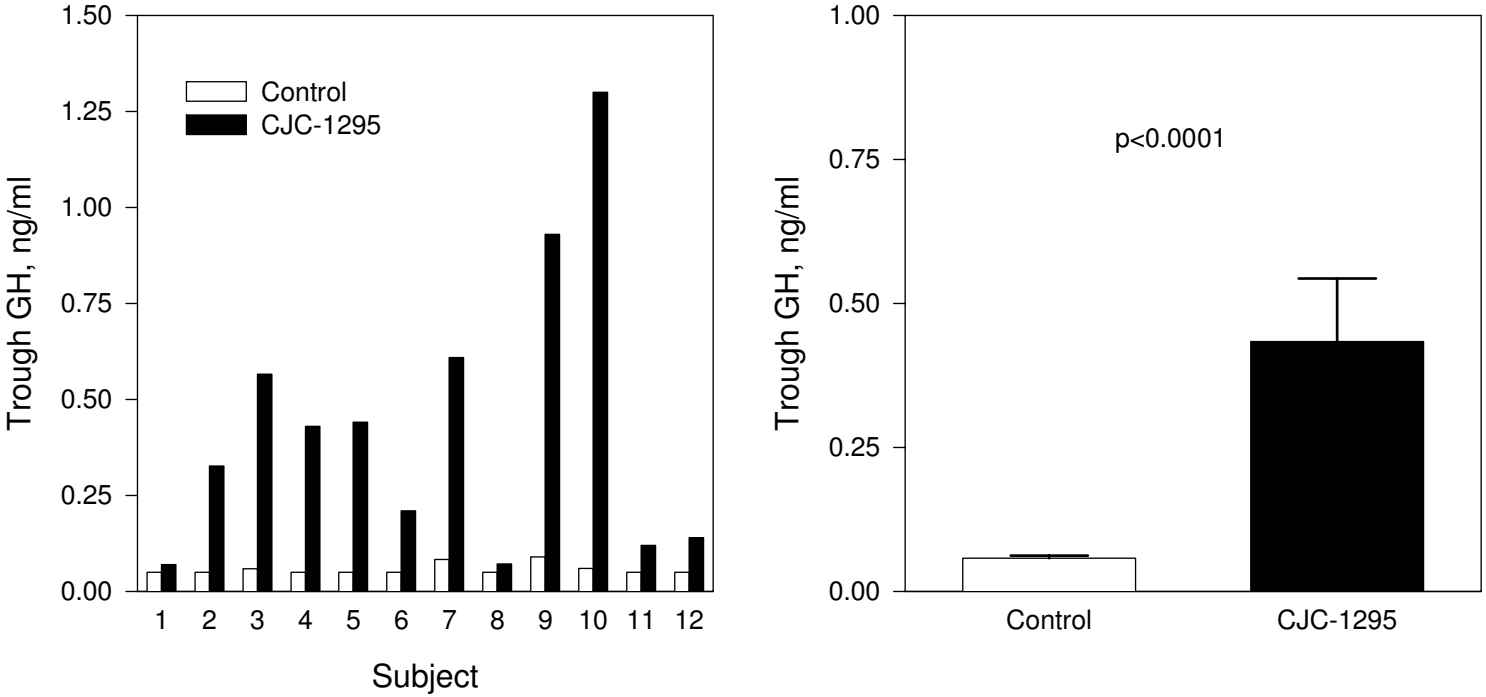

1.50
1.25
1.00
0.75
0.50
0.25
0.00
Trough GH, ng/ml
Control
CJC-1295
1 2 3 4 5 6 7 8 9 10 11 12
Subject
1.00
0.75
0.50
0.25
0.00
Trough GH, ng/ml
p<0.0001
Control
CJC-1295

Figure 3

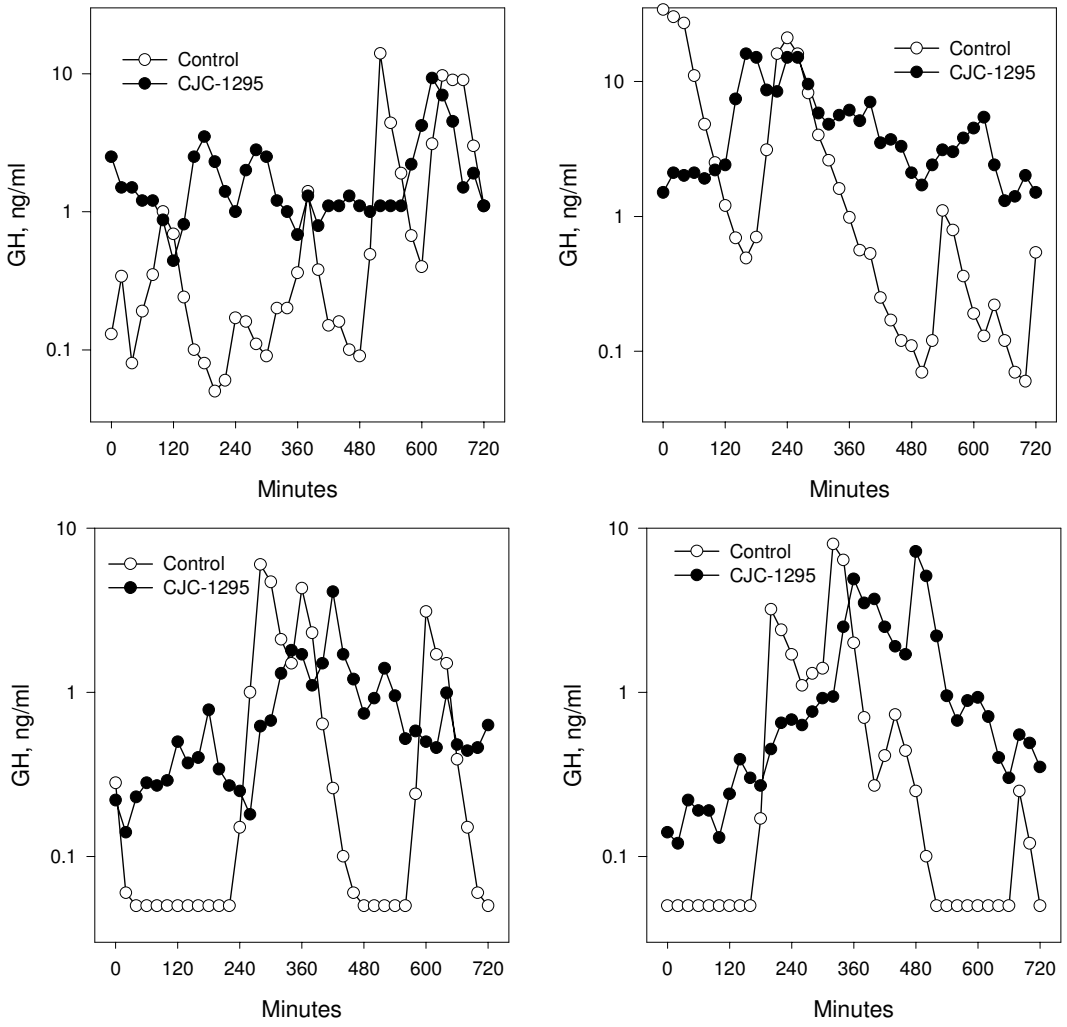
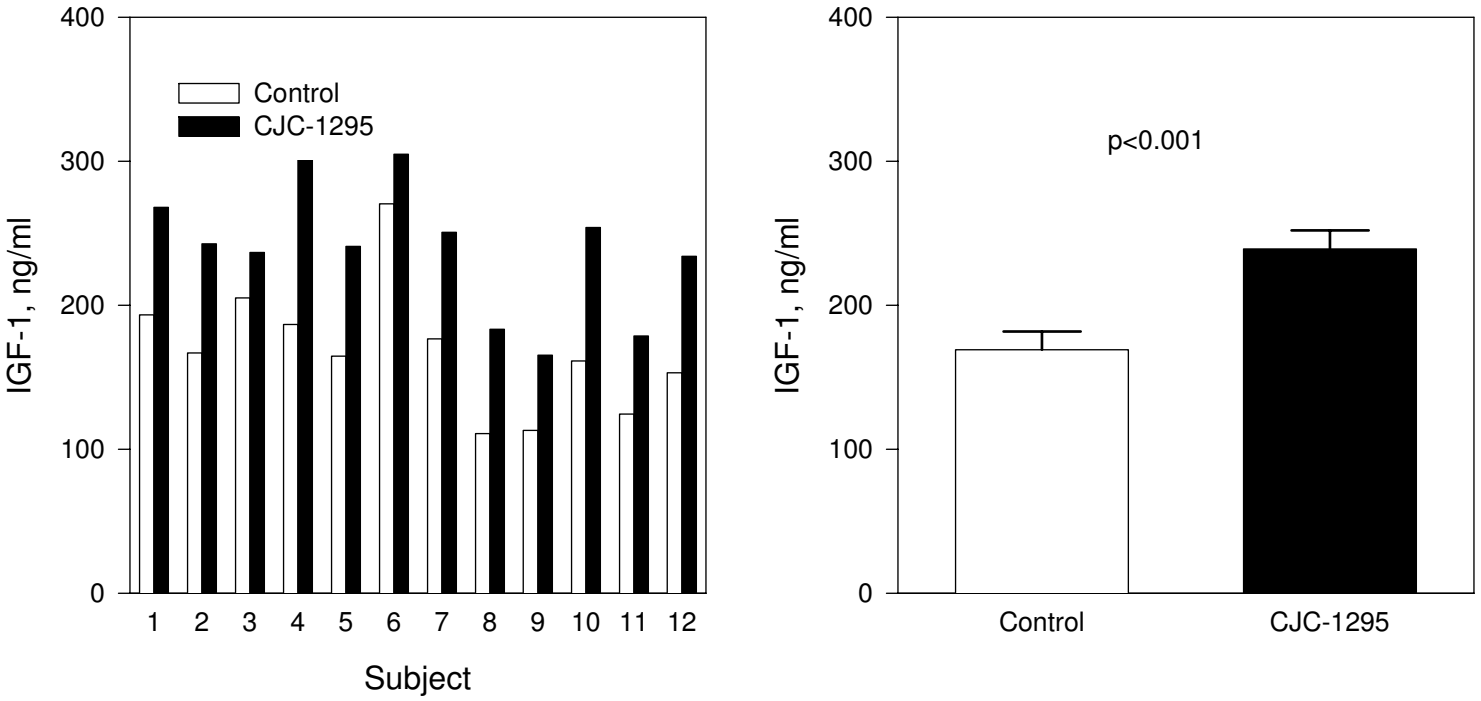

Control
CJC-1295
GH, ng/ml
10
1
0.1
0
120
240
360
480
600
720
Minutes

Figure 4


400
300
200
100
0
IGF-1, ng/ml
Control
CJC-1295
1 2 3 4 5 6 7 8 9 10 11 12
Subject
400
300
200
100
0
IGF-1, ng/ml
p<0.001
Control
CJC-1295