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The effect of caloric restriction interventions on growth hormone secretion in non-obese men and women

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Summary

Lifespan in rodents is prolonged by caloric restriction (CR) and by mutations affecting the somatotropic axis. It is not known if CR can alter the age-associated decline in GH, IGF-1 and GH secretion.

Aim—To evaluate the effect of caloric restriction on GH secretory dynamics.

Methods—Forty-three young (36.8±1.0y), overweight (BMI 27.8±0.7) men (n=20) and women (n=23) were randomized into four groups; Control=100% of energy requirements; CR=25% calorie restriction; CR+EX=12.5% CR+12.5% increase in energy expenditure by structured exercise; LCD=low calorie diet until 15% weight reduction followed by weight maintenance. At baseline and after six months, body composition (DXA), abdominal visceral fat (CT) 11-h GH secretion (blood sampling every 10 min for 11 hours; 2100h-0800h) and deconvolution analysis were measured.

Results—After six months, weight (Control:−1±1%, CR:−10±1%, CR+EX:−10±1%, LCD: −14±1%), fat mass (Control:−2±3%, CR:−24±3%, CR+EX:−25±3%, LCD:−31±2%), and visceral fat (Control: −2±4%, CR:−28±4%, CR+EX:−27±3%, LCD:−36±2%) were significantly (p<.001) reduced in the three intervention groups compared to control. Mean 11-h GH concentrations were not changed in CR or control but increased in CR+EX (p<.0001) and LCD (p<.0001) because of increased secretory burst mass (CR+EX: 34±13%, LCD: 27±22%, p<0.05) and amplitude (CR+EX: 34±14%, LCD: 30±20%, p<0.05) but not to changes in secretory burst frequency or GH half-life. Fasting ghrelin was significantly increased from baseline in all three intervention groups however total IGF-1 concentrations were increased only in CR+EX (10±7%, p<0.05) and LCD (19±4%, p<0.001).

Conclusion—A 25% CR diet for 6 months does not change GH, GH secretion or IGF-1 in non-obese men and women.

Keywords

caloric restriction; GH; IGF-1; aging

The somatotropic axis which consists of the pituitary-derived growth hormone (GH) and insulin-like growth factor 1 (IGF-1) undergoes progressive changes with age. Beginning in young adulthood, GH and IGF-1 decline exponentially (Finkelstein *et al.* 1972; Zadik *et al.*

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1985) leading to GH deficiency in many older individuals (Veldhuis 2008). In epidemiological studies, declining GH concentrations correlate with weight gain, reduced insulin sensitivity, dyslipidemia and sarcopenia at a later age (Corpas *et al.* 1993). The strongest correlates of the age-related decline in GH are increased abdominal visceral fat and sex-steroid depletion (Weltman *et al.* 1994; Iranmanesh *et al.* 1998; Erickson *et al.* 2005; Veldhuis *et al.* 2005a; Veldhuis *et al.* 2005b).

Across the lifespan, studies of GH secretion indicate that reduced availability of IGF-1 is mostly explained by decreased GH secretion (Iranmanesh *et al.* 1994). In comparison to young adults, aged individuals have small GH pulses or reduced GH secretion per burst. Basal GH release, maximal GH secretion, the number of secretory bursts and elimination kinetics (Iranmanesh *et al.* 1994; Gentili *et al.* 2002; Veldhuis *et al.* 2005c) do not appear to be affected by aging. Furthermore aging does not impair GH stimulated hepatic IGF-1 production (Arvat *et al.* 1998; Lissett & Shalet 2003).

GH replacement therapy gained interest as an anti-aging treatment after a six month study in elderly men showed promising changes in body composition (Rudman *et al.* 1990). A growing database of over 220 patients (age 69±6 years) across 18 randomized controlled trials (Liu *et al.* 2007), now suggests that only minor improvements in body composition are observed (small decreases in fat mass and increases in lean mass) in conjunction with a high prevalence of adverse events such as edema and impaired glucose tolerance.

Caloric restriction (CR), a non-pharmacological intervention of reduced energy intake, increases health span and lifespan in most animal species and has been advocated as an anti-aging intervention in humans. An alteration in neuroendocrine function is one of the proposed explanations for delayed aging and longevity in rodents undergoing CR (Fontana 2009). Serum IGF-1 concentrations are reduced up to 4-fold in CR fed rodents in parallel to lifespan extension. Similarly manipulation of the GH-IGF-1 axis (or signaling pathway) leading to lower IGF-1 concentrations in *ad libitum* fed rodents extends lifespan (Flurkey *et al.* 2001; Ikeno *et al.* 2003; Bonkowski *et al.* 2006). Since the blunted somatotropic axis in obesity can be restored by long-term CR and/or weight loss (Williams *et al.* 1984; Rasmussen *et al.* 1995) we hypothesized that like in rodents, a decline in neuroendocrine function may also be characteristic of CR in humans. The aim of our study was to evaluate for the first time changes in GH secretory dynamics after six months of CR in non-obese humans.

Results

There was an equal distribution of males and females within the four groups and no group differences were observed at baseline for anthropometric or body composition characteristics.

Body weight and composition changes (Figure 1)

As previously reported (Redman *et al.* 2007), body weight, FM, FFM and visceral adipose mass were reduced from baseline in all three intervention groups and remained stable in the control group. By design, body weight and composition were stabilized in the LCD group between months 3 and 6, while both continued to decrease in CR and CR+EX. After six months body weight was significantly reduced in CR (−10.4±0.9%), CR+EX (−10.0±0.8%) and LCD (−13.9±0.7%) compared to the control group (−1.1±0.9%) and each intervention group had significant losses of fat mass (CR:−24±3%, CR+EX:−25±3%, LCD:−32±3%) and fat-free mass (CR:−5±1%, CR+EX: −3±1%, LCD −6±1%). Abdominal visceral fat was significantly reduced in the three intervention groups compared to control (Control:−2±4%, CR:−28±4%, CR+EX:−27±3%, LCD:−36±2%).

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GH Secretion

At baseline we did not observe any differences in 11-h mean GH concentrations, GH secretion or fasting IGF-1 and ghrelin concentrations between groups (Table 1). Table 2 summarizes the changes in GH secretion dynamics after 6 months of intervention in the four treatment groups. The control group had no significant changes from baseline for any of the measured variables. For the CR group, there were no changes in mean 11-h GH concentrations or GH secretion after 6 months of calorie restriction. There were however significant increases in ApEn (21±14%, p=0.04) and in the apparent half life (11±5%, p=0.02) of GH. In the CR+EX group we did observe a significant increase in 11-h mean GH concentration by 41±8% (p<.0001) mostly due to increases in both pulse amplitude (34±14%, p=0.02) and pulse mass (34±14%, p=0.04). 11-h mean GH concentrations were increased by 53±8% (p<.0001) in the LCD group after 6 months of the intervention due to an increase in secretory burst mass (28±22%, p<0.05) and amplitude (30±20%, p<0.01) without changes in the frequency of secretory bursts, interburst interval or GH half-life.

Ghrelin and IGF-1

In response to 6 months of caloric restriction, fasting ghrelin concentrations were significantly increased (p<0.03) from baseline in all three intervention groups but not in the control group: 7±1% in CR; 7±3% in CR+EX; and 8±3% in LCD. Caloric restriction significantly increased total IGF-1 concentrations in CR+EX (10±7%, p<0.05) and LCD (19±4%, p<0.001) only (Table 2).

Discussion

A progressive decline in GH often leading to somatopause, or relative GH deficiency, is a characteristic of normal aging. CR is an intervention postulated to delay the onset of age-related disease and increase mean and maximal lifespan. One of the proposed theories by which CR promotes longevity in rodents, is via an inhibition of the somatotropic axis and GH-IGF-1 signaling pathways. In this randomized controlled trial of CR in overweight humans we characterized changes in GH secretory dynamics before and after 6 months of CR. On its own, a 25% CR diet did not modulate GH concentrations, GH secretion or IGF-1 concentrations. Interestingly CR in conjunction with exercise (CR+EX) and a low calorie diet followed by weight maintenance (LCD) increased GH concentrations by more than 40%, altered GH secretion and increased serum IGF-1.

In rodents, CR is one of the most robust experimental strategies known to delay aging and extend lifespan. Concentrations of IGF-1 are reduced in proportion to the level of CR (Hursting *et al.* 1993; Dunn *et al.* 1997; Berrigan *et al.* 2002) and lower levels of IGF-1 have been associated with the anti-carcinogenic benefits of CR in rodents (Anisimov 2001). Furthermore, genetic mutants of GH and GH-resistance display a delayed aging phenotype and an extended lifespan (Bartke 2005). The lifespan of growth hormone-receptor knockout mice resembles that of normal mice subjected to a 30% CR diet (Bonkowski *et al.* 2006). Similarly the Laron dwarf mouse, produced by a targeted disruption of the growth hormone receptor, is GH resistant and long lived (Bartke & Brown-Borg 2004). Dwarf mice (Ames and Snell), which lack growth hormone live longer than normal siblings and display many signs associated with delayed aging (Bartke *et al.* 2001; Flurkey *et al.* 2001; Bartke & Brown-Borg 2004). These genetically modified mice that suffer defects in the production of GH or IGF-1 or in responsiveness to GH that is, they express significantly lower levels of circulating IGF-1, have decreased onset of age-associated cancers (Hursting *et al.* 2003).

Most of the metabolic benefits of CR associated with longevity in rodents however are also observed in humans (Heilbronn *et al.* 2006). In both humans and rodents, CR leads to

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increased insulin sensitivity (Larson-Meyer *et al.* 2006), a lowering of the metabolic rate and core body temperature (Heilbronn *et al.* 2006), decreased DNA damage (Heilbronn *et al.* 2006) and inflammation (Lefevre *et al.* 2009) and reduced concentrations of insulin and triiodothyronine (Heilbronn *et al.* 2006). Our finding in this randomized trial of CR supports the recent observation that IGF-1 levels are not decreased in individuals self imposing nutritious CR diets for 6 years (Fontana *et al.* 2008), suggesting that long-term CR diets also may not modulate the GH:IGF-1 axis in humans. Collectively these observations indicate that CR in humans, at least over the short term, does not involve changes GH or IGF-1 and that the metabolic improvements of CR observed in humans occur independent of the somatotropic axis. Chronic CR such as anorexia nervosa however, does increase GH secretion and reduce IGF-1 concentrations (Argente *et al.* 1997; Scacchi *et al.* 1997) however this is likely due to severe under-nutrition.

The results from our study cannot fully explain why GH secretion was increased with the CR+EX and LCD interventions. Declining levels of growth hormone mostly attributed to impairment in pulsatile GH secretion are negatively associated with body fatness. Centrally, a blunted response of GH secretion is observed with pharmacological stimulation at both the hypothalamus (Topper *et al.* 1984; Kopelman *et al.* 1985) and pituitary levels (Williams *et al.* 1984; Kopelman & Noonan 1986). Diet-induced weight loss (Williams *et al.* 1984; Tanaka *et al.* 1990; Rasmussen *et al.* 1995) and even fasting in obese subjects have been shown to normalize GH secretion and GH response to growth hormone stimulating hormone (Kelijman & Frohman 1988). Weight loss alone in our 6 month study does not fully support the previously reported changes in GH. Body weight and body fatness were reduced in the three intervention groups and to the same degree in the CR and CR+EX groups. The larger reduction in body weight and body fatness in the LCD group may be a potential explanation for the greatest improvement in GH. However, body weight and composition changes alone cannot explain why an increase in GH was not observed in the CR group. It should be noted that by design, our participants were overweight and not obese. Visceral adiposity is a strong negative predictor of GH secretion (Clasey *et al.* 2001). The change in visceral fat may be involved in the increased GH response but is unlikely to be the sole factor. Visceral fat was reduced by ~30% in the CR+EX group and also in the CR group where GH secretion was unchanged. The LCD group however had the most marked changes in GH secretion and the greatest loss of visceral fat.

In addition to body composition changes, the different response of GH secretion to our 3 CR diets draws attention to the possible roles of ghrelin, IGF-1 and exercise. In the current study, ghrelin, a GH secretagogue, is probably not mediating the increase in GH secretion since ghrelin concentrations were increased in all the intervention groups but not to a greater extent in CR+EX and LCD where GH and IGF-1 increase.

It is well known that a single bout of exercise increases GH secretion from pre-exercise levels (Sutton & Lazarus 1976; Friedmann & Kindermann 1989) and exercise training has been shown to increase 24h GH secretion in young obese women (Weltman *et al.* 1992). In our study, the inconsistent findings between CR and CR+EX despite similar weight losses and similar body composition changes could reflect an increased GH secretion due to exercise training. In an earlier study, exercise training over one year however was not a stimulus for GH secretion in older adults (Hartman *et al.* 2000) which was attributed to a lack of change in adiposity (Wideman *et al.* 2002).

The IGF-1 data are less clear. In obesity, the role of IGF-1 in the blunted GH axis is debated. Generally obese individuals have normal total IGF-1 levels, and caloric restriction in normal weight (Fontana *et al.* 2008) or overweight subjects (Walford *et al.* 2002; Fontana *et al.* 2008) does change IGF-1 concentrations. IGF-1 concentrations are reduced in proportion to

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decreased dietary protein (Fontana *et al.* 2008). In our study, total IGF-1 concentration was not altered by CR alone, but was increased in the LCD and CR+EX groups. We did not have sufficient serum available to measure concentrations of IGF-1 binding proteins, which are important for the estimation of free IGF-1 and further interpretation of these findings.

In summary, 25% CR in overweight but non-obese young humans did not increase GH secretion or change IGF-1 concentrations over 6 months. This finding questions the involvement of the somatotropic axis in nutritionally sound CR diets in humans. Calorie restriction in combination exercise and a low calorie diet with weight loss greater than 10% however, increased GH secretion. We were unable to explain the non-uniform findings between the three CR interventions (CR, CR+EX, LCD) by changes in body fatness, visceral adiposity, ghrelin or IGF-1. Further studies are needed to determine whether longer interventions of CR alone can reverse the age-associated decline in the somatotropic axis or if the addition of exercise is necessary to retain youthful GH secretion.

Experimental Procedures

Subjects

The study was approved by the Pennington Biomedical Research Center IRB and the Data Safety Monitoring Board of CALERIE and subjects provided written informed consent prior to participating. Forty-six healthy, overweight ($25 \geq BMI < 30$) men (25 – 50 y) and pre-menopausal women (25 – 45 y) completed the study. Participants were excluded if they smoked, exercised more than twice per week, were pregnant, lactating or post-menopausal, had a history of obesity (BMI>32), diabetes, cardiovascular disease, eating disorders, psychological disorders, substance abuse or regularly used medications except for birth control. Details of the screening process and study population have been extensively described (Heilbronn *et al.* 2006). The physical characteristics of the participants during weight maintenance at baseline are summarized in Table 1.

Study design

In brief, participants were randomized into one of four groups for 24 weeks: control = healthy weight maintenance based on AHA Step 1 diet, CR = 25% caloric restriction from baseline energy requirements, CR+EX = 12.5% caloric restriction and 12.5% increase in energy expenditure through structured aerobic exercise and LCD = low calorie diet (890kcal/d) to achieve a 15% reduction in body mass followed by weight maintenance. The group assignment was stratified to ensure the equal distributions of sex and BMI (Table 1).

Energy Requirements

As detailed previously (Redman *et al.* 2009), the energy intake required for weight maintenance during the baseline testing and the subsequent energy deficit prescribed during the intervention were calculated from 4-week data including two 14-day periods by doubly labeled water (DLW). During the first DLW study participants followed their usual diet at home (B1) whereas they were provided with a weight maintenance diet adjusted to maintain weight within 0.25 kg during the second DLW study (B2). Individual energy requirements were calculated as the average of total daily energy expenditure (TDEE) during B1 and energy intake for weight maintenance during B2.

Diet and behavioral intervention

During weeks 1–12 and 22–24 of the intervention, participants were provided with all meals which were prepared by the metabolic kitchen at the Center and based on individual energy intake targets. During weeks 13–22 participants self-selected a diet based on their individual targets. The diet composition was based on the American Heart Association guidelines, 30%

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calories from fat, 15% from protein and 55% from carbohydrate. During self-selected feeding, compliance to the dietary intervention was monitored from weekly self-reported food records and changes in body weight. Participants attended weekly behavioral group or individual sessions and cognitive-behavioral techniques were used to not only teach subjects how to adhere to their meal and exercise plans but to boost motivation to the demanding study interventions.

Exercise Prescription and Compliance

Participants in CR, LCD and Control were required to continue their usual pattern of physical activity while participants in CR+EX increased their energy expenditure by 12.5% above baseline through structured aerobic exercise, five days per week. At least three of the five weekly exercise sessions were performed under supervision at the center. The exercise program was implemented gradually and by week six all participants were expending the required 12.5% of baseline energy expenditure. Exercise energy expenditure was initially determined by indirect calorimetry and thereafter heart rate was used to assess compliance during all exercise sessions. On average, the target energy cost was maintained at 403±63 kcal per session for women and 569±118 kcal per session for men resulting in an average exercise duration of 53±11 min and 45±14 min per session, respectively.

Physiological Testing

All physiological testing (Heilbronn *et al.* 2006) was conducted during a 5-day inpatient stay in the inpatient unit at baseline and during weeks 12 and 24 of intervention. Following a 12h fast and morning void, percent body fat was measured using DXA (Hologics QDR 4500A, Bedford, MA) and fat mass (FM) and fat-free mass calculated. Abdominal fat distribution was measured by multi-slice computed tomography (GE Light Speed, General Electric, Milwaukee, WI) as previously described (Larson-Meyer *et al.* 2006).

Sampling Protocol and hormone assays

On the 4^{th} inpatient day, frequent blood samples were obtained from an intravenous catheter placed in an anticubital vein for 24 hours for the determination of leptin circadian rhythm (not reported) and GH pulsatility. From 0800h until 2100h samples were collected at 30 minute intervals after which the frequency was increased to every 10 minutes until 0800h the following morning. Participants were not permitted to sleep during the day. Standard meals calculated on the basis of calorie levels were consumed at 0900h (breakfast), 1130h (lunch) and 1700h (dinner). Water was consumed ad libitum. Lights were turned off from 2030h to 0700h. Samples were kept on ice and processed every 30 minutes with aliquots of serum frozen at −80°C until measured in a single batch. Serum GH concentrations were determined by chemiluminescent immunoassay using the Immulite 2000 instrument (Siemens Healthcare Diagnostics, Deerfield, IL); intra- and inter- assay coefficient of variation (CV) were 3.7% and 5.7%, respectively. Serum IGF-1 concentrations were measured by ELISA (Diagnostic System Laboratories, Inc. Webster, TX); intra- and inter-assay CVs were 6.0% and 6.7%, respectively. Total ghrelin concentrations were determined in plasma by radioimmunoassay (Linco Research Inc., St Charles, MO); intra- and interassay CVs were 6.4% and 16.3%, respectively.

Deconvolution analysis

Biexponential deconvolution analysis was used to quantitate basal and pulsatile secretion from the hormone concentration profiles (Veldhuis *et al.* 1987). In view of parameter interdependence, estimation was conditioned statistically on mean rapid-phase kinetics and *a priori* pulse times (Veldhuis *et al.* 1995). The directly measured rapid-phase half for GH was 3.5 min with a fractional amplitude of 0.37 (Faria *et al.* 1989). Putative GH pulse times

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were determined by Cluster (discrete peak-detection) analysis at a nominal $P < 0.05$ false-positive rate, as validated from simulations and empirical data (Veldhuis & Johnson 1986; Urban *et al.* 1989). Rapid-phase kinetics and peak positions were held constant, allowing valid simultaneous estimation of the amplitude and half-duration (and thereby mass or integral) of secretory bursts, slow-phase half-life and underlying basal secretion (initially estimated as corresponding to the lowest 5% concentration). The outcomes are basal (time-invariant), pulsatile (sum of secretory-burst mass) and total (sum of basal and pulsatile) secretion expressed in hormone concentration units per time.

Approximate entropy (ApEn) analysis

ApEn is a regularity statistic (Pincus 1991; Pincus & Goldberger 1994), which measures relative orderliness. Elevated ApEn denotes greater irregularity of subpattern evolution over time, which, in turn, signifies loss of feedback and/or feedforward control (Hartman *et al.* 1994; Pincus *et al.* 1999; Veldhuis *et al.* 2001). Studies have demonstrated the validity, reliability and utility of ApEn to discriminate regularity of hormone patterns with high sensitivity [> 90%] and specificity [> 90%] (Pincus *et al.* 1999; Veldhuis *et al.* 2001).

Statistical Analysis

Data in text and tables are provided as means ± SEM. SAS Version 9.12 (SAS Institute, Cary, NC) was used for analysis. The changes in all variables from baseline to month 6 were computed and data were analyzed by ANCOVA with treatment and time interactions and baseline values as covariates. $P<0.05$ was considered statistically significant.

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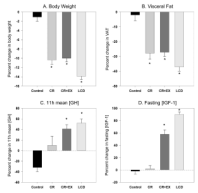

Figure 1.
The effect of 6 month of caloric restriction on body weight (panel A), visceral fat (panel B), 11-h mean growth hormone (panel C) and total IGF-1 (panel D) concentrations. * Significant changes from baseline (p=0.05).

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Table 1

Subject characteristics and G H secretory dynamics at baseline. Data are summarized as mean ± sem

	Control N=11	CR N=11	CR+EX N=12	LCD N=9	Treatment Effect, p-value
Gender (F/M)	6/5	5/6	7/5	5/4	
Age, *years*	37.7±2.2	38.4±1.6	35.5±1.6	38.1±2.6	0.62
Weight, *kg*	81.0±2.7	82.6±3.1	81.7±3.2	81.4±3.6	0.99
11-h mean concentration, *ug/L*	1.02±0.66	0.93±0.63	1.06±0.66	1.09±0.79	0.95
Total production rate, *ug/L·11-h*	33.0±22.8	37.4±32.2	37.6±26.1	45.4±36.1	0.81
ApEn	0.49±0.19	0.43±0.14	0.41±0.17	0.45±0.13	0.67
GH Half life, *min*	19.13±2.15	17.79±3.07	19.09±1.48	20.31±2.58	0.12
Number of bursts per 11-h	10.6±4.5	10.0±2. 6	11.9±3.6	10.8±2.1	0.56
Burst Amplitude, *ug/L·min*	0.17±0.10	0.18±0.14	0.17±0.12	0.23±0.22	0.74
Burst Mass, *ug/L*	3.0±1.6	3.6±2.9	3.4±2.5	4.3±3.6	0.75
Interburst interval, *min*	52.5±16.0	60.4±19.1	58.1±15.8	61.7±11.5	0.17
Fasting total IGF-1, *ug/L*	424.3±37.9	418.0±28.4	380.1±34.8	408.4±62 .2	0.74
Fasting total Ghrelin, *pmol/L*	2095±105	2357±137	2277±230	2723±198	0.18

Control=healthy weight maintenance diet, CR=25% caloric restriction from baseline energy requirements, CR+EX=12.5% caloric restriction and 12.5% increase in energy expenditur through structured aerobic exercise and LCD=low calorie diet (890kcal/day) to achieve a 15% weight loss followed by weight maintenance.

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Table 2

Changes in GH concentration and GH secretion dynamics after 6 months of calorie restriction (6 month - baseline)

	CR	CR+EX	LCD	Control
11-h mean concentration, *ug/L*	0.15±0.16	0.83±0.21*	1.47±0.41*	−0.33±0.11
Total production rate, *ug/L·11-h*	1.3±10.8	31.2±11.9*	32.8±12.6*	−12.9±4.5
Approximate Entropy (ApEn)	0.20±0.09*	−0.03±0.05	0.02±0.08	0.04±0.06
GH Half life, *min*	2.49±0.95*	0.43±0.79	0.61±0.51	0.65±0.88
Number of bursts per 11-h	3.2±1.9	0.0±1.1	1.1±0.9	0.9±0.4
Burst Amplitude, *ug/L·min*	0.00±0.03	0.16±0.06*	0.19±0.09*	−0.07±0.02
Burst Mass, *ug/L*	−0.18±0.71	3.00±1.16*	3.70±1.89*	−1.17±0.49
Interburst interval, *min*	−3.1±17.0	6.4±8.5	−1.8±6.7	3.1±7.6
Fasting total IGF-1, *ug/L*	2.0±20.9	57.7±30.2*	89.8±19.5*	−1.9±20.4
Fasting total Ghrelin, *pg/mL*	187.5±26.8*	250.0±31.6*	91.0±52.6*	20.6±26.9

*Significant changes from baseline (p=0.05).