Provocative Stimulation of Growth Hormone: A Monozygotic Twin Study Discordant for Spinal Cord Injury

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Abstract

Background/Objective: A blunted growth hormone (GH) response to provocative testing and/or low levels of plasma insulin-like growth factor-I (IGF-I) have been reported in persons with spinal cord injury (SCI). A reduction in activity of the GH-IGF-I axis may have deleterious effects on body composition and function. Provocative testing for GH stimulation was performed to determine the response in monozygotic twins that were discordant for SCI.

Methods: GH stimulation testing was performed by the administration of intravenous arginine.

Results: Nine SCI twins with paraplegia, a mean age of 39 ± 9 years, and duration of injury of 14 ± 9 years were studied. The twins with SCI had a significantly lower body mass index than non-SCI twins (22.5 ± 4.0 vs 25.1 ± 4.2 kg/m²; $P < 0.05$); percent fat mass was greater in the twins with SCI ($30 \pm 11\%$ vs $22 \pm 10\%$; $P < 0.05$). Baseline serum GH was correlated with percent fat only in the SCI twins. The response to GH provocative stimulation was less in the twins with SCI: peak GH response was 5.8 ± 6.6 vs 13.0 ± 7.3 ng/mL ($P < 0.05$), and sum GH response was 15.7 ± 15.6 vs 30.2 ± 17.3 ng/mL ($P = 0.06$). Although baseline serum GH was correlated with stimulated response in the SCI twins, this relationship was not found in the non-SCI twins. Adiposity was positively related to the provocative serum GH response in twins with SCI rather than negatively related, as noted in the non-SCI twins.

Conclusions: This study confirms and extends prior work that reported a reduction in stimulated GH release in persons with SCI, which was related to baseline values.

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Key Words: Spinal cord injuries; Growth hormone; Insulin-like growth factor-I; Paraplegia; Arginine stimulation test; Monozygotic twins

INTRODUCTION

Persons with spinal cord injury (SCI) are generally less active than able-bodied individuals, and they are relatively more obese per unit body weight (1-3). In a previous study by our group, subjects with SCI had a blunted growth hormone (GH) response to provocative stimulation; in addition, younger individuals with SCI had lower levels of insulin-like growth factor (IGF)-I compared with an able-bodied control group (4). Shetty et al (5) noted that plasma levels of insulin-like growth factor-I (IGF-I) were depressed in those with SCI (5). Depressed

levels of plasma IGF-I in poliomyelitis survivors have been correlated with greater dysfunction in activities of daily living (6). When performing a cross-sectional, comparison study, the experimental group is often matched, but this "matching" is never optimal. As a component project of a study to determine changes in body composition and hormonal milieu in monozygotic twins, discordant for SCI, we performed provocative testing with intravenous arginine for GH stimulation. We hypothesized that this study would confirm and extend prior work that found a reduction in GH release in persons with SCI.

METHODS

Subjects

Nine pairs of monozygotic twins (8 male pairs and 1 female pair), discordant for SCI, were recruited for study.

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Figure 1. The GH response to intravenous arginine. Solid squares, subjects with SCI; gray circles, non-SCI subjects. Each point is expressed $\pm$ SE.

All SCI twins had paraplegia, were not able to bear weight, and used a manual wheelchair for mobility. At the time of study, all subjects were in good health. None of the subjects with SCI were receiving any of the following medications: major or minor tranquilizers, calcium channel blockers, α -adrenergic agonists, β -adrenergic antagonists, or cholinergic antagonists.

Procedures

Genetic testing for zygosity was performed in all pairs at 6 independent, highly polymorphic loci (Lifecodes, Stamford, CT). All 9 pairs were found to be strongly consistent with monozygosity, with a probability of being nonidentical of 1 in 4,096. Institutional Review Board approvals from the James J. Peters Veterans Affairs Medical Center, Bronx, NY, and the Mount Sinai School of Medicine, New York, NY, were obtained before initiating the study. The subjects were provided with verbal and written information about the study, and written consent was obtained.

After an overnight fast, arginine hydrochloride (30 g/subject) was infused intravenously into an antecubital vein over 30 minutes. Blood was collected at 0, 30, 60, 90, and 120 minutes. Radioimmunoassay was performed for plasma GH (ICN Pharmaceuticals, Costa Mesa, CA) on the samples collected before and after arginine administration. Peak GH response is defined as the highest value

Figure 2. The peak and sum GH responses to intravenous arginine. Symbols above bars display significant differences between the SCI and non-SCI groups: * $P < 0.05$, $^{\dagger}P = 0.06$. Solid bars, SCI twins; gray bars, non-SCI twins. Values are $\pm$ SE.

attained during the test. The sum GH response is the summation of the 5 GH values from time 0 to time 120 minutes. Plasma IGF-I was measured at baseline by radioimmunoassay (Nichols Institute, San Jan Capistrano, CA). To exclude other endocrine abnormalities, baseline determinations for thyroid function tests, testosterone, estradiol, and cortisol were performed by kit radioimmunoassay (ICN Pharmaceuticals); luteinizing hormone (LH) and follicle-stimulating hormone (FSH) were performed by kit radioimmunoassay (Diagnostic Products Corporation, Los Angeles, CA).

Height was measured in the standing position for the non-SCI twins, and body length was determined lying in the supine position for the SCI twins. Weight was measured on a Weight-Tronix scale (Scale Electronics Development, New York, NY). Body mass index (BMI) was calculated as body weight in kilograms divided by height in meters squared. The soft tissue compartment of fat mass was measured by dual-energy x-ray absorptiometry (DXA) on a total body scanner (model DPX, Lunar Radiation, Madison, WI) (7,8).

Table 1. Subject Characteristics

Characteristics	SCI		Non-SCI		P Value
	Mean $\pm$ SD	Min, Max	Mean $\pm$ SD	Min, Max	
Age (years)	39 $\pm$ 9	25, 58	39 $\pm$ 9	25, 58	NS
DOI (years)	14 $\pm$ 9	2, 25	...	...	...
BMI (kg/m ²)	22.5 $\pm$ 4.0	17.0, 30.3	25.1 $\pm$ 4.2	18.6, 30.3	0.03
Percent fat	29.9 $\pm$ 10.9	11.0, 44.0	21.8 $\pm$ 10.3	9.0, 38.0	0.005

DOI, duration of SCI; NS, not significant.

Table 2. Stimulated Results

Stimulated Values	SCI		Non-SCI		P Value
	Mean $\pm$ SD	Min, Max	Mean $\pm$ SD	Min, Max	
Sum GH (ng/mL)	15.7 $\pm$ 15.6	4.9, 53.6	30.2 $\pm$ 17.3	10.1, 64.7	0.06
Peak GH (ng/mL)	5.8 $\pm$ 6.5	2.2, 22.0	13.0 $\pm$ 7.3	3.0, 23.5	0.03
Time to PKR (min)	30 $\pm$ 26		60 $\pm$ 26		0.05

PKR, peak response.

Statistical Analyses

The results are expressed as mean $\pm$ SD, except in Figures 1 and 2, where SE of the mean was used. A paired *t* test was performed between the SCI and non-SCI twins for weight, percent fat, BMI, and all other continuous outcome variables. Because there was a significant group by time interaction effect, a repeated-measures analysis of variance (ANOVA) was performed for the stimulated GH response, and the time-points for group differences in GH response were determined by ANOVA and paired *t* tests at specific time-points (eg, at 0, 30, 60, 90, and 120 minutes). Linear regression analysis was performed between peak or GH with BMI and percent fat.

RESULTS

The mean age of the twins was 39 $\pm$ 9 years. The average duration of injury in the twins with SCI was 14 $\pm$ 9 years, with a median duration of 11 years (range, 2–25 years). The twins with SCI had a lower BMI than the non-SCI twins, and percent fat was greater in the twins with SCI (Table 1). The response to provocative stimulation was less in the twins with SCI (Figures 1 and 2). There was a significant group by time interaction effect ($F = 3.63$, $P < 0.01$). The peak GH and sum GH responses were blunted in the twins with SCI (Table 2; Figure 2). The time to peak response was shorter in the twins with SCI compared with the non-SCI twins (Table 2). In the SCI twins, but

not in the non-SCI twins, a significant correlation was evident between baseline and stimulated serum GH values (baseline vs sum GH: $r = 0.82$, $P < 0.005$; baseline vs peak GH: $r = 0.87$, $P = 0.001$). Plasma IGF-I was similar in both groups (Table 3).

In twins with SCI, a correlation was found between baseline GH and percent fat ($r = 0.67$, $P < 0.05$); there was a trend for the stimulated GH response to correlate directly with percent fat (Figure 3). In non-SCI twins, negative correlations approaching significance ($P = 0.06$) were found between stimulated serum GH values and percent fat (Figure 3). There was no significant relationship between IGF-I and measures of adiposity for the SCI (IGF-I vs BMI: $r = -0.21$, not significant; IGF-I vs percent fat: $r = -0.31$, not significant) or non-SCI twins (IGF-I vs BMI: $r = -0.25$, not significant; IGF-I vs percent fat: $r = 0.15$, not significant).

Baseline endocrine studies were within normal limits and were not significantly different between SCI and non-SCI twins (Table 3). Of note, total serum testosterone was correlated with the peak GH response in non-SCI twins ($r = 0.68$, $P < 0.05$), but this relationship was not evident in twins with SCI ($r = 0.61$, not significant).

DISCUSSION

Basal or random GH sampling may not provide an adequate differentiation between individuals who are GH

Table 3. Baseline Hormone Levels

Baseline Hormone Levels	SCI		Non-SCI		P Value
	Mean $\pm$ SD	Min, Max	Mean $\pm$ SD	Min, Max	
GH (ng/mL)	2.2 $\pm$ 0.9	1.4, 4.3	1.9 $\pm$ 0.5	1.4, 2.8	NS
IGF-1 (ng/mL)	263 $\pm$ 78	153, 392	260 $\pm$ 54	191, 344	NS
Tot T (ng/mL)	3.7 $\pm$ 2.0	0.2, 6.4	4.0 $\pm$ 2.3	0.2, 8.2	NS
FSH (MIU/mL)	6.9 $\pm$ 5.7	2.9, 21.0	6.7 $\pm$ 3.9	2.5, 13.5	NS
LH (MIU/mL)	3.5 $\pm$ 0.9	2.5, 5.2	3.6 $\pm$ 1.0	2.5, 5.8	NS
E ₂ (pg/mL)	72 $\pm$ 22	32, 110	91 $\pm$ 33	58, 170	NS
T ₃ (ng/dL)	108 $\pm$ 20	82, 139	104 $\pm$ 12	81, 121	NS
T ₄ (μ g/dL)	7.5 $\pm$ 1.5	5.4, 9.3	7.4 $\pm$ 1.2	5.5, 9.1	NS
TSH (μ U/mL)	1.3 $\pm$ 0.6	0.7, 2.4	1.3 $\pm$ 0.8	0.55, 3.14	NS
Cortisol (μ g/dL)	14.6 $\pm$ 7.2	9.2, 31.8	16.1 $\pm$ 4.5	10.6, 23.9	NS

Tot T, total testosterone; E₂, estradiol; T₃, triiodothyronine; T₄, thyroxine; TSH, thyroid-stimulating hormone.

Figure 3. The relationship between percent fat and stimulated GH responses in SCI and non-SCI twins. (A and B) SCI twins: percent fat with sum and peak serum GH values ($r=0.58$, $P=0.10$ and $r=0.58$ and $P=0.11$, respectively). (C and D) Non-SCI twins: percent fat with sum and peak serum GH values ($r=-0.64$, $P=0.06$ and $r=-0.64$, $P=0.06$, respectively).

deficient from those with a normal pattern of release. As such, provocative testing has been shown to provide a better means to determine inadequate pituitary GH release. Intravenous arginine is appreciated to be a potent stimulus for GH release (9). The work presented herein confirms earlier studies by us and others that persons with SCI have suboptimal response to provocative GH testing (4–6,10). Huang et al (11) also showed that persons with SCI had a reduced and/or delayed response to provocative stimulation with GH-releasing hormone and insulin-induced hypoglycemia.

In a cross-sectional study, persons with chronic SCI had a greater reduction in lean tissue above the level of lesion—that is, in the arms and trunk—compared with able-bodied controls; interestingly, the age-related loss of lean tissue in the legs was parallel in controls and those with chronic SCI; albeit leg lean tissue in persons with SCI

was markedly reduced across all ages (1). These results raise the question as to whether some of the adverse body composition of SCI may be the result of a less than favorable hormonal milieu, in part, because of a reduction in circulating GH. Although the absolute fat mass may not be increased, persons with SCI have been shown to be relatively more obese than able-bodied individuals (1,2). Spungen et al (1) noted that those with SCI were on average 13% fatter per unit BMI than controls. In this study, twins with SCI had significantly more body fat mass than their non-SCI co-twins. In the able-bodied population, an increase in body adiposity is appreciated to reduce GH release (12,13), and a negative relationship was found herein in our non-SCI twins between body adiposity and peak or sum GH release. In contrast, in those with SCI, a trend was noted for a direct relationship between stimulated GH values and

body adiposity, which is inexplicable. In SCI twins, a significant relationship was found between baseline serum GH values and the stimulated response, as is often observed with other provocative hormone responses. It is not clear why plasma IGF-I levels were not different between the groups, despite the significant blunting in response to provocative GH stimulation in the twins with SCI. Possibly, because of the limited number of twin pairs studied and perhaps because they were relatively young, a difference between the groups may have been difficult to show. Of note, a far stronger within-pair correlation for plasma IGF-I levels has been reported in monozygotic than dizygotic twin pairs (14).

GH has appreciated anabolic effects on muscle. Several studies in the elderly have shown a beneficial effect of GH administration on body composition (15–18). IGF-I has trophic effects on muscle in animal models (19,20). As determined by absolutely low levels of plasma somatomedin C (eg, plasma IGF-I), Shetty et al (5) reported that 46% of 41 men with tetraplegia had little or no GH secretion compared with 24% in 119 able-bodied controls. Of note, in another study in poliomyelitis survivors living in the community, plasma IGF-I levels were low and correlated with greater difficulty with activities of daily living, such as needing increased assistance, increased difficulty in dressing or driving, and increased pain with activities (6). Thus, lower plasma IGF-I levels seemed to have an adverse effect on neuromuscular function and quality of life.

GH is available by parenteral administration, but the cost of therapeutic administration is prohibitive. Administration of GH in adults has been associated with increased fluid retention and nerve entrapment syndromes, most commonly carpal tunnel syndrome (21,22). In prior work, we have shown that individuals with SCI who are prescribed baclofen have higher plasma IGF-I levels and a greater response to provocative stimulation than those not treated with the drug (10). Recently, our group described that low-dose oral baclofen, a GABAergic agent, administered in a prospective fashion over several weeks caused an increase in plasma IGF-I levels (23). Although these findings are intriguing and tempt clinical application, more study is needed before recommending baclofen administration to favorably effect the GH-IGF-I axis, as well as potential salutary effects on body composition and function, in persons with SCI.

CONCLUSION

A few studies have investigated the GH-IGF-I axis in persons with SCI. In prior studies, there was depression of pituitary GH release either with provocative stimulation or a decrease of plasma IGF-I levels or both. Our work herein confirms and strengthens these findings by showing that twins with SCI have markedly reduced GH release after provocative testing compared with the non-SCI co-twins. In contrast to the non-SCI twins, there seems to be a trend toward greater GH release after

provocative testing directly related to body adiposity in the twins with SCI. Of note, in those with SCI, baseline serum GH values were positively related to adiposity. The changes in body composition that have been previously reported in persons with SCI suggest that perhaps a hormonal mechanism may be operative because there is greater loss in lean body tissue above the level of lesion and relatively increased adiposity in those with SCI compared with able-bodied controls. The effect of pharmacologically increasing levels of circulating GH or IGF-I on body composition, function, and independence in persons with SCI has not yet been studied.

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