Growth Hormone, Insulin, and Insulin-like Growth Factor-1 in Hypermobility Syndrome

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ABSTRACT. ***Objective.*** To investigate growth factors in patients with hypermobility syndrome (HMS), namely insulin, insulin-like growth factor-1 (IGF-1), and growth hormone (GH).

Methods. Standard radioimmunoassay quantified serum levels of insulin, IGF-1, and GH in 24 women and 7 men with HMS, and in suitable age and sex matched controls. Several patients with other heritable connective disorders were also studied, including congenital hip dysplasia and severe kyphosis.

Results. Patients with HMS and with otherwise unexplained joint and muscle pain were found to have elevated levels of insulin, IGF-1, and GH. Patients with heritable connective tissue disorders had elevated GH levels, and several patients had elevated insulin and IGF-1 levels.

Conclusion. In patients with HMS, elevation of serum growth factors helps establish the diagnosis. GH alone can induce muscle and joint pain. (J Rheumatol 2001;28:1666–9)

Key Indexing Terms:
GROWTH HORMONE INSULIN INSULIN-LIKE GROWTH FACTOR-1
HYPERMOBILITY HERITABLE CONNECTIVE TISSUE DISORDERS

The earliest description of the hypermobility syndrome (HMS) is attributed to Hippocrates, who described markedly lax joints in Scythians in the 4th century BC[1]. Scythians were the forebears of modern inhabitants of Eastern Europe. The Scythians not only recognized the disorder, which caused a loss of important skills such as throwing the javelin and archery, but even devised an effective treatment, scarification of the involved joints. Two Russian patients provided case material for the first detailed clinical description of this syndrome, known in the West as Ehlers-Danlos syndrome and in the East as Chernogubov's syndrome[2]. Although HMS became a generally recognized clinical syndrome following the paper by Kirk, *et al* in 1964[3], it remains a clinical challenge to rheumatologists. Concomitants of hypermobility may vary in both children and adults from trivial to serious, encompassing nonarticular as well as musculoskeletal aspects[4]. Additional reports indicate growth hormone (GH) has arthritogenic properties as evidenced by articular manifestation of acromegaly and the observation that acromegalic arthropathy is a reversible rheumatic disorder[5].

Patients with HMS are set apart from others with arthritis by the spectrum of lesions occurring in the individual over many years. We sought to correlate the physical findings of joint hypermobility with the serum level of GH, insulin-like growth factor-1 (IGF-1), and insulin. Perturbations in these neurohormones occur in several rheumatic disorders — osteoarthritis (OA)[6], diffuse idiopathic skeletal hyperostosis (DISH)[7], and acromegalic arthropathy[5]. In this study group, other rheumatic disorders were excluded as a cause of associated musculoskeletal symptoms by appropriate clinical and laboratory evaluation.

MATERIALS AND METHODS

The study was conducted in the Arthritis Clinics of University Hospitals of Cleveland and Wade Park Veterans Administration Hospital in Cleveland. Institutional review boards of both hospitals approved the study. Participants were all volunteers, aged 20 years or more.

Our criteria for the diagnosis of HMS were based on commonly accepted physical findings of Bulbena, *et al*[8]. Additionally, Grahame[9] found that extensibility of the 5th metacarpophalangeal joint over 90° was the most valuable and sensitive finding in evaluation of patients. Beighton[10] devised a scoring system for hypermobility. At any age, females are more hypermobile than males. To study our patients in broader physiologic context (including their history of childbearing) we devised a questionnaire (Table 1). For diagnosis we required 2 or more arthrodial features (part A, Table 1) and one or more systemic features (part B). No other researcher has used these criteria. Onset of clinical symptoms due to or related to hypermobility is difficult to standardize. Patients and parents do not interpret the symptoms associated with HMS uniformly. Patients were excluded if they were diabetic, alcoholic, pregnant, had psoriasis, or were using oral contraceptives, vitamin A medication, or corticosteroids. Muscle and joint pain was judged due to hypermobility when diagnostic investigation failed to identify any of the known connective tissue disorders. Healthy age and sex matched controls were recruited from hospital personnel screened to exclude those with any systemic features of HMS as well as other musculoskeletal disease.

Several individual patients with other heritable connective tissue disorders were studied with the diagnosis based on clinical features as outlined in the *Primer on the Rheumatic Diseases*[11] and matched by age and sex

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(Table 3). Scoliosis, kyphosis, and mitral valve prolapse are considered to have an association with HMS.

Methods. Blood from patients and from controls was drawn by venipuncture for concomitant assays of glucose, IGF-1, insulin, and GH. Blood was clotted at room temperature, then centrifuged and serum was removed. Aliquots were separated and stored at –70°C until assayed. Samples were used if the glucose was in the euglycemic range, 65–130 mg/dl. This basal metabolic state was attained after an overnight fast or a fast of ≥ 4 h. Hyperglycemic and hypoglycemic samples were excluded because these variations might affect insulin levels.

Standard radioimmunoassays (RIA) (Incstar, Stillwater, MN, USA) were adapted for IGF-1, insulin, and GH. The manufacturer provided standard control specimens for each peptide with each kit. A standard curve and control specimen were evaluated with each run. Normal values for pertinent demographic groups were determined for our laboratory including white women and black women. All subjects were over 20 years of age.

IGF-1 assay is a double antibody disequilibrium RIA that includes octadecasilylsilica column extraction of serum sample. After extraction, the RIA is performed by adding sample and rabbit anti-IGF-1 serum, followed by incubation for 2 h. Then ^{125}I-IGF-1 tracer preprecipitated carrier is added and incubated for 20 h; then goat anti-rabbit precipitating complex (2nd antibody) with propylene glycol (PEG) is added in one step (Incstar manual for IGF-1 No. 53065). After 2 h in the cold, the solutions and the centrifuge supernate are poured off and residual precipitate counted. IGF-1 values vary with age.

Control studies gave the following results: coefficient of variance (CV), intrakit 10 assays, 8%; interkit CV 10 assays, 5.6%. Sensitivity of the method was defined as the apparent concentration at 3 standard deviations at maximum binding. The minimum detectable amount is 2.0 nM/l. The specificity is as follows: 1.0% cross reactivity for the following peptides, IGF-II, human GH, fibroblast growth factor, transforming growth factor, and platelet derived growth factor.

The insulin assay begins with the removal of endogenous insulin antibodies and other interfering substances by treatment of the serum with PEG. The assay is an equilibrium assay in which first antibody and tracer are combined and incubated for 16 to 20 h. We selected this method as being more sensitive than the 3 h recommended by the manufacturer (Incstar manual for insulin No. 06130). Glucose was measured with each serum insulin assay. Since insulin secretion is influenced by many endocrine and non-endocrine disorders as well as dietary factors, serum samples were used only when blood glucose measured 65–130 mg/dl. Standard control sera were provided by the manufacturer with every assay to assure validity of assay results. A quality control graph is included with each kit giving the performance characteristics obtained by the manufacturer's quality control laboratory using the reagent lots included in that particular kit. Quality control studies gave the following results: intrakit CV, 10 assays, 12%; interkit CV, 10 assays, 8.5%. This insulin antibody has the following cross reactivity: human and porcine insulin 100%, proinsulin 30%, human C-peptide of insulin 0.01%. The minimum detectable amount is 2.0 μU/ml.

The GH assay is a disequilibrium RIA using addition of sample and guinea pig anti-human serum followed by incubation. ^{125}I-GH is then added, followed by a second incubation. Then prepercipitate carrier, second antibody, and PEG are added in a single step (Incstar manual for human GH, No. 07130).

Since GH levels fluctuate because of many factors, we chose to study our patients and controls at basal physiologic levels as defined by a normoglycemic state after overnight fasting. Nearly all samples, patient and control, were drawn between 8:30 A.M. and noon. A fasting specimen is recommended by the manufacturer but not required. In the normal fasting person, the baseline for GH approaches zero. Our GH assay was adapted for low values by plotting the standard curves on a log-logit paper that produces a straight line and using a Beckman 5500 gamma counter attached to a computer, which prints the lowest detectable value.

Standard control sera are provided in every assay to insure the validity of each assay's results. The Incstar GH standard has been calibrated against the World Health Organization standard 66/127. These values are printed on the quality control graph included in each kit. Our evaluation for GH RIA for low to medium values gave the following: intrakit CV, 10 assays, 6.8%; interkit CV, 10 assays, 10.5%. Cross reactivity of the GH antibody was 0.8% with each of the following human peptides: insulin, prolactin, beta-endorphin, follicle stimulating hormone, luteinizing hormone, thyroid stimulating hormone, chorionic gonadotrophin, placental lactogen, and adrenocorticocotrophin. The minimal detectable amount is 0.4 ng/l.

Glucose was measured by the highly specific hexokinase method (Sigma, St. Louis, MO, USA). Other hexoses such as fructose and mannose are detected but they are present only in trace amounts. Quality control evaluation gave the following results: intrakit CV, 10 assays, 8%, interkit CV, 10 assays, 8%. For evaluation of data, we utilized a standard technique comparing groups of different sizes, the 1-tailed Student t test, with $p < 0.05$ indicating significance.

Table 1. Criteria for clinical assessment of hypermobility syndrome.

A. Arthrodial features
   1. Knee, elbow hyperextension — passive ≥ 10°
   2. Fingers — metacarpophalangeal (MCP) "clicking" on pulling of finger, "dimpling" of skin over MCP
   3. Thumb — passive opposition of thumb to the flexor aspect of forearm
   4. Passive hyperextension of fingers to lie parallel to extensor surface of forearm
   5. Passive dorsiflexion of 5th finger ≥ 90°
   6. Scapular winging
   7. Muscle or joint pain — in absence of known connective tissue disorder
   8. History of dislocation of patella, shoulder, or other

B. Systemic features
   1. Skin — velvety, hyperextensible, especially posterior axillary area, eyelid hyperextensible, flat scars, few or no stretch marks following pregnancy
   2. Palate — high arch or presence of epulis
   3. Obstetric history — prematurity in patient or immediate family, involuntary sterility, relative sterility — only one pregnancy
   4. Blue sclerae
   5. Ecchymoses with minimal trauma, bleeding, platelet dysfunction
   6. Mitral valve prolapse
   7. Scoliosis

RESULTS

Female patients with HMS (n = 24) had statistically higher serum levels of GH, IGF-1, and insulin than did age and sex matched controls (Table 2). A smaller group of male patients (n = 7) had significantly elevated serum levels of GH and insulin compared to controls. The male patients had higher serum IGF-1 levels than controls, but perhaps due to the small sample size, differences did not reach significance. Analysis of variance among the groups indicated no further adjustment was required since the random distribution followed that for a normal population.

Elevated serum GH levels were seen in 10 of 10 patients (6 female, 4 male) (Table 3) with heritable connective tissue disorders, distributed as follows: 2 had congenital hip dysplasia with hip pain; one was a proportionate dwarf with knee pain; one patient with Marfan syndrome had knee pain; a patient with homocysteinuria had back pain, scoliosis, and osteoarthritis (OA) in the lumbar spine. The pain in the knee reported by the patient with both OA and Marfan syndrome may have been in part due to OA and in part to Marfan syndrome. A patient with mitral valve prolapse had elevated GH and insulin levels; she had no symptoms. The youngest

Table 2. Serum growth hormone (GH), insulin-like growth factor (IGF-1), and insulin in hypermobility syndrome.

Group	n	Age, yrs	GH, ng/ml	IGF-1, nM/l	Insulin, U/ml
Females					
Control	20	39 ± 13	0.94 ± 0.03	20.0 ± 7	5.9 ± 2
HMS	24	36 ± 10	2.73 ± 2	26.8 ± 10	15.3 ± 10
p		NS	< 0.001	< 0.009	< 0.003
Males					
Control	24	49 ± 15	0.78 ± 0.2	18.4 ± 5	7.5 ± 3
HMS	7	57 ± 12	1.31 ± 0.2	19.3 ± 2	11.4 ± 4
p		NS	< 0.001	< NS	< 0.02

Values are mean ± SD. NS: not significant.

Table 3. Insulin growth hormone (GH), and insulin-like growth factor-1 (IGF-1) in other heritable connective tissue disorders.

Number/Sex	Diagnosis	Age, yrs	GH ng/ml	IGF-1, ng/ml	Insulin. μU/ml
1. F	Marfan syndrome, OA knees and knee pain	33	2.49*	26.0*	—
2. F	Congenital hip dysplasia, hip pain	28	13.7*	19.1	8.3*
3. F	Congenital hip dysplasia, hip pain	46	13.4*	25.2*	4.0
4. F	Proportionate dwarfism, knee pain	28	23.0*	22.5*	5.2
5. F	Homocysteinuria, scoliosis, OA lumbar spine, back pain	37	2.33*	15.2	—
6. F	Mitral valve prolapse (no joint symptoms)	63	1.81*	22.9*	11.2*
7. M	Severe kyphosis (no symptoms)	74	0.96*	15.9	4.9
8. M	Severe kyphosis (no symptoms)	52	1.05*	25.6*	13.9*
9. M	Pseudo-ainhum (constricting bands on all phalanges, appearance similar to ainhum ichthyosis, a parasitic infection)	58	1.18*	15.4	—
10. M	Scheuermann's disease, back pain, kyphosis	20	2.48*	52.8*	—

*Elevated compared to normal levels (mean ± SD), as follows:

	Age, yrs	GH, ng/ml	IGF-1, ng/ml	Insulin, μU/ml
Female controls (n = 20)	39 ± 13	0.94 ± 0.3	20.0 ± 7.5	5.9 ± 2
Male controls (n = 24)	49 ± 15	0.78 ± 0.2	18.4 ± 5	7.5 ± 3

patient, with Scheuermann's syndrome, had the highest GH level. Serum IGF-1 levels were elevated in 4 women, and insulin was elevated in 2 women.

All 4 male patients had elevated GH. Two with severe kyphosis had no symptoms; another had less severe kyphosis with back pain. Two men had elevated levels of IGF-1; 2 were in normal range.

Elevation of serum GH in patients who had pain in joints established an association of joint pain with elevation of GH.

DISCUSSION

Multiple perturbations involving growth factors occur in HMS and heritable connective tissue disorders. Aberrations in growth factors found in our patients with HMS (namely, elevation in GH, IGF-1, and insulin) differ from those we reported in patients with OA[6], in whom GH and insulin were increased, while IGF-1 was decreased. Similarly, patients with DISH show elevated growth factors[7]. Denko and Malemud[12] reported that GH is found in the red cells of patients with OA, where its functions include storage and transport. The red cell and serum levels in individual patients are variable and may relate to the clinical activity.

Textbooks of biochemistry and physiology have taught that GH stimulates cartilage growth. But it also inhibits cartilage metabolism in normal rats receiving radioactive sulfate as a marker[13].

Animal models contribute to our understanding of the functions of GH. Multiple functions of GH are revealed in cartilage biology using radioactive sulfate as the metabolic marker. In rats rendered more sensitive to GH by pituitary removal, GH injections result in increased uptake of ^{35}S in cartilage[13]. In normal intact rats receiving injections of GH, the cartilage showed inhibition of ^{35}S incorporation[13].

Reinhart and Li[14] induced experimental arthritis with properties of OA and rheumatoid arthritis in animals by injections of hypophyseal GH. Berczi and co-workers[15] could not induce adjuvant arthritis in hypophysectomized animals unless GH or prolactin was added. Prolactin is similar to GH in stimulating incorporation of radioactive sulfate[16].

Dalsgaard and co-workers[17] report that individual neurons contain multiple neuropeptide messengers, including growth factors that, in connective tissue, stimulate cell growth and acute and chronic inflammation. We have shown that in patients with active OA, muscle and joint pain are associated with excess GH[6].

In acromegalic arthropathy[5], joint pain and stiffness are controlled only when serum GH levels are reduced to normal. In patients with OA, excess serum GH is present when symptoms are present. When clinical symptoms are controlled, we found that serum GH is at normal levels[18]. Thus patients with OA may have intermittent periods of "excess" GH when symptoms are active, normal GH levels when symptoms are controlled. Thus pathologic findings in OA are associated with excess GH interfering with normal physiology.

It is noted that all our patients with heritable connective tissue disorders had elevated GH; several had, in addition, elevated IGF-1 and/or insulin. In these patients no growth factor was found to be decreased. This phenomenon is of interest and should be explored further.

When red cells release their GH, the serum GH, now elevated, may stimulate the sensitive connective tissue to respond by inducing arthritic symptoms.

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