# Decreased levels of IGF binding protein-3 in serum from children with Perthes' disease

Tomoko Matsumoto[1], Hiroshi Enomoto[1], Katsuro Takahashi[1] and Satoru Motokawa[2]

**The concentrations of insulin-like growth factor (IGF)-I and IGF-binding protein (IGFBP)-3 in serum obtained from 27 children with Perthes' disease and 10 age-matched control subjects were measured by radioimmunoassay (RIA). IGFBPs were also analyzed by a Western ligand blotting (WLB) method. The bone age was determined in 18 patients from hand-wrist radiographs. Serum levels of IGFBP-3 but not IGF-I were significantly lower than those in normal controls. WLB showed a decrease in the intensity of the IGFBP-3 band in 19 of the 27 patients. The bone age was delayed, 2 years or more compared with the chronological age in 7 of 18 patients, and all of them, except 1, showed decreased levels of IGFBP-3 on WLB. We conclude that there may be disturbances in availability of IGFs in some patients with Perthes' disease.**

Department of Orthopedic Surgery, [1]Nagasaki University, School of Medicine, 1-7-1, Sakamoto, Nagasaki, 852, Japan
Tel +81 95 849-7321. Fax -7325 [2]Nagasaki-Chuo National Hospital
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Some children with Perthes' disease have short stature without any endocrinological disturbances. This indicates that there may be some factor affecting the growth of bone and cartilage tissues in this disease. Insulin-like growth factors (IGFs) promote the differentiation and proliferation of cartilage tissue (Isaksson et al. 1991, Seong et al. 1994). There have been some reports regarding IGFs in the serum of patients with Perthes' disease but the findings of these studies are contradictory (Burwell et al. 1986, Kitsugi et al. 1989, Neidel et al. 1992). In serum, most IGFs are bound to IGF-binding proteins (IGFBPs) and are carried to target cells. Six IGFBPs have been found and cloned (Shimasaki and Ling 1991), but the roles of each IGFBP have not been clarified. IGFBP-3 is the most abundant in normal serum, forming a 150 kilodalton (kDa) triple complex with an acid-labile subunit, which prolongs the biological half-life of IGFs (Baxter et al. 1989). We postulated that there might be impaired bioavailability and bioactivity of IGFs together with a concomitant alteration of IGFBPs in Perthes' disease.

## Patients and methods

Serum samples were obtained from 27 children (24 boys) having Perthes' disease with a mean age of 8 (6–11) years, and from 10 healthy control subjects (8 boys) with a mean age of 8 (6–11) years. After acid/ethanol extraction, IGF-I was measured by a radioimmunoassay (RIA) (Miell et al. 1992) using a commercially available human IGF-I kit developed by the Nichols Institute Product (San Juan Capistrano, CA), which is supplied by Eiken Chemical Co. (Tokyo, Japan). No cross-reactivity with IGF-II and insulin was detected. IGFBP-3 was measured by RIA (Blum et al. 1990), the reagents for which were purchased from Diagnostic Systems Laboratories (Webster, Texas). Cross-reactivity with the other IGFBPs was less than 0.4%. IGFBPs in serum were analyzed by Western ligand blotting (WLB), as previously described (Hossenlopp et al. 1986, Matsumoto et al. 1996). Briefly, 10 µL serum were electrophoresed on 10% SDS-polyacrylamide gel, and the size-fractionated proteins were then electroblotted onto nitrocellulose membrane. After incubating overnight with [$I^{125}$] IGF-II at 4 °C, the IGFBPs were visualized by autoradiography. Bone age was determined on hand-wrist radiographs (Sugiura 1985).

### *Statistics*

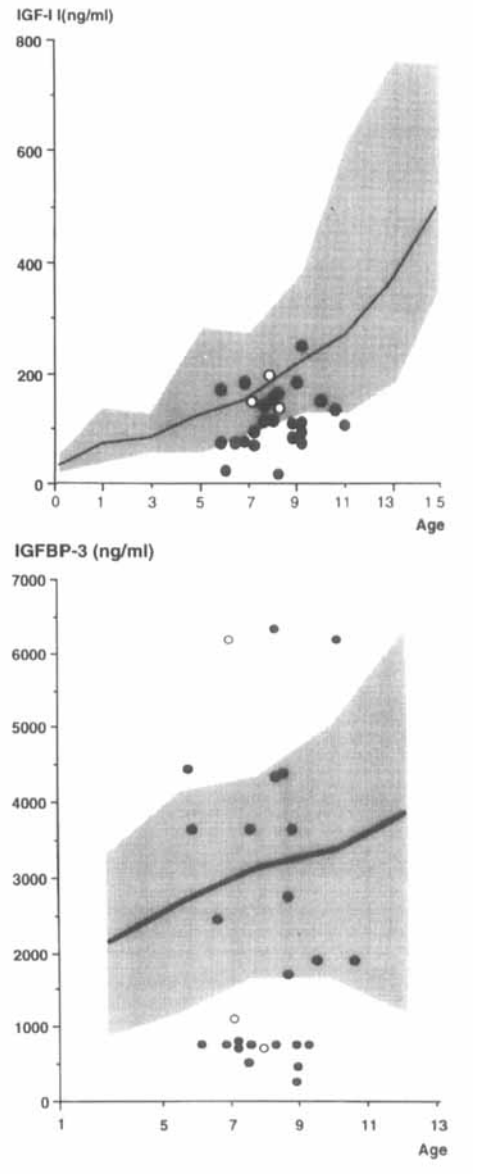
Results are expressed as mean (SD). Significance was assessed by the nonparametric Mann-Whitney test. P-values less than 0.05 were considered significant.

## Results

The IGF-I serum level was 119 (55) ng/mL in Perthes' disease and 176 (113) ng/mL in the control group (Table, $p = 0.2$). IGFBP-3 serum levels in Perthes'

**Data of 27 patients with Perthes' disease and 10 controls. Serum levels of IGF-I and IGFBP-3 were measured by radioimmunoassay. The amount of IGFBP-3 band detected by Western ligand blotting is shown as normal (→),moderately reduced (↓), and severely reduced (↓ ↓), compared to the controls. ND not determined**

| Case | Sex | Chronol. age | Skeletal age | IGF-I (ng/mL) | IGFBP-3 (ng/mL) | IGFBP-3 by WLB |
|---|---|---|---|---|---|---|
| *Patients* | | | | | | |
| 1 | M | 5Y7M | 5Y4M | 20 | 850 | ↓↓ |
| 2 | M | 6Y1M | 4Y | 158 | 3750 | ↓ |
| 3 | M | 6Y1M | ND | 69 | 4500 | ↓↓ |
| 4 | M | 6Y7M | 4Y5M | 198 | 800 | ↓↓ |
| 5 | M | 7Y1M | 5Y | 59 | 800 | ↓↓ |
| 6 | M | 7Y3M | 4Y1M | 10 | 550 | ↓↓ |
| 7 | M | 7Y3M | ND | 99 | 2500 | → |
| 8 | F | 7Y4M | 7Y4M | 198 | 6250 | → |
| 9 | M | 7Y4M | 7Y2M | 158 | 4450 | ↓ |
| 10 | M | 7Y5M | ND | 69 | 900 | → |
| 11 | F | 7Y5M | 7Y | 149 | 1250 | ↓ |
| 12 | M | 7Y6M | 5Y | 109 | 900 | → |
| 13 | M | 7Y7M | ND | 69 | 900 | → |
| 14 | M | 7Y8M | 6Y5M | 149 | 4450 | ↓ |
| 15 | M | 8Y1M | ND | 129 | 6500 | ↓ |
| 16 | M | 8Y4M | ND | 119 | 3750 | ↓ |
| 17 | F | 8Y5M | ND | 158 | 800 | ↓ |
| 18 | M | 8Y6M | 6Y3M | 198 | 350 | ↓↓ |
| 19 | M | 8Y9M | 8Y | 89 | 1750 | ↓ |
| 20 | M | 8Y10M | 7Y4M | 109 | 600 | ↓ |
| 21 | M | 8Y11M | 7Y | 109 | 3750 | → |
| 22 | M | 9Y | 7Y4M | 79 | 900 | ↓↓ |
| 23 | M | 9Y1M | 7Y | 158 | 2000 | ↓ |
| 24 | M | 9Y1M | 7Y5M | 89 | 850 | ↓↓ |
| 25 | M | 9Y2M | ND | 238 | 2500 | → |
| 26 | M | 11Y6M | ND | 99 | 2000 | ↓ |
| 27 | M | 11Y7M | 12Y | 128 | 6250 | → |
| Mean | | | | 119 | 2402 | |
| SD | | | | 55 | 1953 | |
| *Controls* | | | | | | |
| 1 | F | 6Y | | 188 | 4000 | |
| 2 | M | 6Y | | 223 | 4000 | |
| 3 | M | 6Y | | 103 | 4250 | |
| 4 | M | 7Y | | 119 | 6750 | |
| 5 | M | 8Y | | 94 | 4000 | |
| 6 | M | 8Y | | 149 | 5100 | |
| 7 | F | 9Y | | 74 | 2450 | |
| 8 | M | 11Y | | 114 | 5000 | |
| 9 | M | 11Y | | 238 | 3500 | |
| 10 | M | 11Y | | 455 | 3000 | |
| Mean | | | | 176 | 4205 | |
| SD | | | | 113 | 1205 | |




The serum levels of IGF-I and IGFBP-3 in patients with Perthes' disease. ● boys, ○ girls. Established normal ranges are shown in gray area.

disease 2402 (1953) ng/mL were lower than in the control group 4205 (1205) ng/mL (Table, p = 0.009).

The number of controls is so small that the values obtained for IGF-I or IGFBP-3 were plotted against established ranges in healthy male subjects, which were obtained by measuring the serum levels of IGF-I in 97 healthy boys and the levels of IGFB-3 in 202 healthy boys by using the same kit as in this study (unpublished data, shown in the manufacturers' materials) (Figure). Some patients with Perthes' disease showed low levels of IGF-I or IGFBP-3.

WLB analysis of IGFBPs in normal control serum showed a major doublet of 41 and 39 kilo-dalton (kDa) and 3 other bands of 34, 30 and 24 kDa. These bands were identified as IGFBP-3, -2, -1 and -4, respectively, by immunoprecipitation. The WLB of normal serum showed almost the same pattern, so we pooled these sera as a control, and the amounts of IGFBP-3 in patients were compared to those of the controls. In 19 of the 27 children with Perthes' disease, the density of the IGFBP-3 band was moderately or severely reduced. Serum IGFBP-3 levels ob-

tained by RIA corresponded well with the density of the IGFBP-3 band on WLB in some patients, however, there were some discrepancies between them (Table).

Bone ages, compared with the chronological ages, determined in 18 of 27 patients, showed a delay of 2 years or more, in 7 patients (Table). In these patients, IGFBP-3 levels (1307 (1198 ng/mL)) were decreased relative to controls ($p = 0.002$). However, IGF-I levels (127 (71 ng/mL)) were within normal limits.

## Discussion

Perthes' disease occurs mainly in young boys. This sexual and age preponderance suggest the presence of general factors. However, no hormonal disturbances have been found, although Rayner et al. (1986) suggested a defect in the pituitary-somatomedin-target tissue axis. Several authors have noted short stature and bone age retardation (Harrison et al. 1976, Kristmundsdottir et al. 1987, Loder et al. 1995), indicating disturbed skeletal maturation in Perthes' disease. IGF-I is one of the most potent factors for growth of bone and cartilage tissue (Isaksson et al. 1991). There have been some reports regarding plasma IGF-I levels in Perthes' disease but the findings have been conflicting (Tanaka et al. 1984, Burwell et al. 1986, Kitsugi et al. 1989, Neidel et al. 1992). One reason may be differences in methods used to measure IGF-I: radio- receptor assay, bioassay or radioimmunoassay. Furthermore, most of these studies paid little attention to the existence of IGFBPs, although more than 95% of IGF-I is bound to IGFBPs in serum. In our study, we measured the plasma levels of IGF-I in patients with Perthes' disease after acid/ethanol extraction to dissociate IGF-I from binding proteins and found that the serum concentrations of IGF-I in children with Perthes' disease were not significantly lower than those in control subjects. This is not in agreement with the findings of Neidel et al. (1992), who employed a similar method to measure IGF-I and found that during the first 2 years after the diagnosis of Perthes' disease, IGF-I plasma levels were reduced.

6 IGF-binding proteins have been characterized and, to date, IGFBP-3 is the most abundant of these in normal serum. In our study, IGFBP-3 serum levels in Perthes' disease were significantly lower than those in controls. These findings are in contrast to those reported by Neidel et al. (1993), which is difficult to explain. Since degraded IGFBP-3 is also detected by RIA, the concentrations of IGFBP-3 do not always reflect the intact IGFBP-3 in serum. Therefore, we examined IGFBPs in serum, using WLB, which measures only the intact form of IGFBP-3. Discrepancies between RIA and WLB measurements of serum IGFBP-3 show the presence of IGFBP-3 protease, which lowers their affinity for IGF-I and may release IGFs from IGFBPs. It is of interest that some patients with Perthes' disease had different patterns of IGFBPs from controls—i.e., a markedly reduced IGFBP-3 band. It is well known that IGFBP-3 is mainly regulated by growth hormone (GH), and therefore GH-deficient children have a reduced IGFBP-3 band (de Boer et al. 1996). The children with Perthes' disease in our study showed no clinical signs of hormonal deficiency; but the concentration of GH was not measured. However, their subsequent growth seemed to be normal. In our study, reduced IGFBP-3 serum levels were related to the reduced IGFBP-3 band in WLB, with a few exceptions, suggesting that the amounts of intact IGFBP-3 are decreased in some patients with Perthes' disease.

## References

Baxter R C, Martin J L, Beniac V A. High molecular weight insulin-like growth factor binding protein complex. Purification and properties of the acid-labile subunit from human serum. J Biol Chem 1989; 264: 11843-8.

Blum W F, Ranke M B, Kietzmann K, Gauggel E, Zeisel H J, Bierich J R. A specific radioimmunoassay for the growth hormone(GH)-dependent somatomedin-binding protein:its use for diagnosis of GH deficiency. J Clin Endocrinol Metab 1990; 70: 1292-8.

Burwell R G, Vernon C L, Dangerfield P H, Hall D J, Kristmundsdottir F. Raised somatomedin activity in the serum of young boys with Perthes' disease revealed by bioassay. Clin Orthop 1986; 209: 129-38.

de Boer H G, Blok G L, Popp-Snijders C, Stuurman L, Baxter R C, Van der Veen E. Monitoring of growth hormone replacement therapy in adults based on measurement of serum markers. J Clin Endocrinol Metab 1996; 81: 1371-7.

Harrison M H, Turner M H, Jacobs P. Skeletal immaturity in Perthes' disease. J Bone Joint Surg (Br) 1976; 58 (1): 37-40.

Hossenlopp P, Seurin D, Segovia-Quinson B, Hardouin S, Binoux M. Analysis of serum insulin-like growth factor binding proteins using Western blotting: use of the methods for titration of the binding proteins and competitive binding studies. Anal Biochem 1986; 154: 138-43.

Isaksson O G P, Ohlsson C, Nilsson A, Isgaard J, Lindahl A. Regulation of cartilage growth by growth hormone and insulin-like growth factor-I. Pathology 1991; 5: 451-3.

Kitsugi T, Kasahara Y, Seto Y, Komai S. Normal somatomedin-C activity measured by radioimmunoassay in Perthes' disease. Clin Orthop 1989; 244: 217-21.

Kristmundsdottir F, Burwell R G, Harrison M H. Delayed skeletal maturation in Perthes' disease. Acta Orthop Scand 1987; 58: 277-9.

Loder R T, Farley F A, Herring J A, Schork M A, Shyr Y. Bone age determination in children with Legg-Calvé-Perthes' disease: A comparison of two methods. J Pediatr Orthop 1995; 15: 90-4.

Matsumoto T, Gargosky S E, Iwasaki K, Rosenfeld R G. Identification and characterization of insulin-like growth factors(IGFs), IGF-binding proteins (IGFBPs), and IGFBP proteases in human synovial fluid. J Clin Endocrinol Metab 1996; 81: 150-5.

Miell J P, Taylor A M, Jones J, Rennie J, Sherwood R, Ross R J M. The administration of human recombinant IGF-I to patients following major gastrointestinal surgery. Clin Endocrinol (Oxf) 1992; 37: 542-51.

Neidel J, Zander D, Hackenbroch M H. Low plasma levels of insulin-like growth factor I in Perthes' disease. A controlled study of 59 consecutive children. Acta Orthop Scand 1992; 63: 393-8.

Neidel J, Schonau E, Zande D, Rutt J, Hackenbroch M H. Normal plasma levels of IGF-binding protein in Perthes' disease. Follow-up of previous report. Acta Orthop Scand 1993; 64: 540-2.

Rayner P H W, Schwalbe S L, Hall D J. An assessment of endocrine function in boys with Perthes' disease. Clin Orthop 1986; 209: 123-8.

Seong S C, Matsumura T, Lee F Y, Whelan M C, Li X Q, Trippel S B. Insulin-like growth factor I regulation of swarm rat chondrosarcoma chondrocytes in culture. Exp Cell Res 1994; 211: 238-44.

Shimasaki S, Ling N. Identification and molecular characterization of insulin-like growth factor binding proteins (IGFBP-1,-2,-3,-4,-5 and -6). Prog Growth Factor Res 1991; 3: 243-66.

Sugiura, Y. Skeletal age of Japanese. Chugai Igaku Co. Tokyo 1985.

Tanaka H, Tamura K, Takano K. Serum somatomedin A in Perthes' disease. Acta Orthop Scand 1984; 209: 124-8.