Low plasma levels of Insulin-like Growth Factor I in Perthes' disease

A controlled study of 59 consecutive children

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We studied Insulin-like Growth Factor I (IGF I) plasma levels, standing height, and weight in 59 consecutive children with Perthes' disease and 59 matched controls. The plasma-IGF I levels, measured by radioimmunoassay after acid ethanol extraction, were reduced in affected children during the first 2 years after the diagnosis of Perthes' disease. Partially paralleling the alterations in IGF I plasma levels, there was a tendency towards growth arrest and impaired weight-gain during early-stage disease, followed by catch-up growth and increased weight-gain. No relation was found between degree of femoral head involvement, according to Catterall (1971), and IGF I plasma-levels or body mass. Our data may reflect an impaired synthesis or release of IGF I relative to age in Perthes' disease, or changes in the affinity or metabolism of IGF binding proteins. The observed changes seem to be of a temporary nature.

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Children with Perthes' disease have been shown to suffer from a general retardation of bone maturation (Fisher 1972, Harrison et al. 1976, Kristmundsdottir et al. 1987), raising the question of a hormonal disorder. The postnatal skeletal development is dependent on pituitary growth hormone (GH), the effects of which are mediated in part by the somatomedins or insulin-like growth-factors (Schoenle et al. 1982, Nilsson et al. 1986, Schlechter et al. 1986, Trippel et al. 1989). The principal agent in this group is somatomedin C/IGF I (Phillips and Vassilopoulou-Sellin 1980), while the role of IGF II in the control of postnatal growth has not been fully clarified. GH-plasma levels seem to be normal in Perthes' disease (Fisher 1972, Tanaka et al. 1984), but contradictory results were reported on the IGFs (Tanaka et al. 1984, Burwell et al. 1986, Rayner et al. 1986, Kitsugi et al. 1989).

We have pursued the hypothesis of an altered metabolism of IGF I in Perthes' disease. Since the yield of IGF-assays is influenced by IGF-binding-proteins (IGF-BP) in human blood (Daughaday et al. 1980), a radioimmunoassay after acid ethanol extraction was used to determine whether IGF I plasma levels in affected children differ from those of normal controls.

Abbreviations: GH growth hormone, IGF insulin-like growth-factor, IGF-BP IGF-binding proteins, RIA radioimmunoassay, Sm somatomedin.

Patients and methods

All 59 children, 48 boys and 11 girls with mean age 7 (3–14) years, who presented to our clinic for Perthes' disease at various stages during the period from January 1, 1988, through January 31, 1991, were included in the study. The diagnosis was confirmed by anteroposterior and lateral radiographs of the hips, and either ^{99}Tc-bone-scan and/or magnetic resonance imaging in all cases. Femoral head involvement was graded by Catterall's method (1971); only radiographs taken at least 6 months after confirmation of the diagnosis were used for grading.

Unilateral Perthes' disease was found in 41/48 boys and 10/11 girls, while both hips were affected in 7/48 boys and 1/11 girls. Mean age at the time of diagnosis was 6 (3–12) years for boys and 5 (2–7) years for girls. 45 children were evaluated within the first 2 years, median 6 (0–22) months, after the diagnosis had been established, while in 14 children the examination was performed later, median 39 (26–88) months (Table 1).

The preliminary control-group consisted of 183 children (94 boys, 89 girls, aged 2–15 years), who had been referred to our department or to three participating colleagues in the Cologne metropolitan area because of minor orthopedic or surgical problems during the same period as stated above. Physical examination revealed no signs of endocrine abnormalities in this group. Since evaluation of the children was performed on an outpatient basis or upon admission to the

Table 1. Data of 59 patients with Perthes' disease and 59 controls, matched for age and sex (chronologic order)

A	B	C	D	E	F	G	H	I	K	L	M	N
1	M	8.3	8.5	R	1	131	35	93	8.3	140	32	417
2	F	2.9	2.4	R	2	91	17	21	3.0	92	15	54
3	M	6.3	15	B	2	125	23	83	6.5	120	21	103
4	M	7.5	6.5	L	2	125	22	91	7.4	128	25	104
5	M	3.6	6.9	B	2	102	16	26	3.7	100	14	45
6	M	4.5	19	B	4	113	18	137	4.6	104	18	168
7	M	7.6	22	L	3	136	43	140	7.8	119	20	192
8	M	6.7	15	B	3	123	25	163	6.7	116	23	205
9	M	7.4	7.0	R	2	113	20	111	7.3	114	18	118
10	M	8.0	26	L	3	124	28	363	8.0	127	29	100
11	M	6.1	0.9	R	3	119	20	92	6.2	114	20	125
12	F	4.2	7.2	L	2	110	19	107	4.1	109	22	153
13	M	7.5	7.2	L	1	130	30	161	7.6	134	26	192
14	M	14	32	R	2	168	59	490	14	158	54	452
15	M	5.7	21	R	3	114	16	32	5.6	112	28	104
16	M	7.7	7.2	L	3	130	25	154	7.9	129	24	236
17	M	11	20	L	2	139	30	112	11	135	31	77
18	M	5.1	1.4	B	4	114	26	87	5.2	112	20	104
19	M	7.8	39	L	4	130	32	300	8.0	121	26	125
20	M	6.2	39	L	2	120	22	105	6.5	115	22	171
21	F	7.5	3.9	L	2	120	22	69	7.7	123	23	160
22	F	6.7	17	R	2	115	20	91	6.7	115	20	192
23	M	4.6	0.4	R	2	110	17	113	4.6	102	15	72
24	M	7.7	0.7	R	2	126	24	69	7.9	129	26	115
25	M	6.9	10	L	2	119	21	116	7.0	122	26	160
26	M	10.9	34	L	4	122	20	103	11	135	47	109
27	M	13	88	R	1	150	34	170	13	142	33	257
28	M	5.9	0.5	L	4	111	17	186	6.1	120	21	219
29	F	4.4	4.1	L	2	107	17	60	4.4	111	18	77
30	M	5.7	13.3	R	4	114	24	65	5.6	113	21	112
31	M	6.2	42	R	2	116	19	69	6.3	121	22	83
32	M	8.1	55	L	2	133	30	174	8.2	130	21	52
33	M	5.8	0.5	R	2	117	22	80	5.9	118	23	96
34	M	11	12	L	4	142	31	204	11	130	27	143
35	F	3.0	8.4	R	1	92	19	80	3.1	93	15	71
36	M	9.2	0.6	R	2	129	28	85	9.2	131	27	259
37	M	6.7	36	L	3	134	31	102	6.7	122	26	145
38	F	5.7	6.0	R	2	116	21	41	5.7	121	31	132
39	M	3.9	6.8	B	4	90	13	102	3.9	102	16	57
40	F	8.4	19	B	2	134	28	165	8.6	135	29	171
41	M	9.7	0.3	L	3	128	28	76	9.6	134	29	244
42	M	7.2	10	L	2	127	24	73	7.2	123	21	46
43	M	3.6	0.9	R	4	99	16	14	3.6	103	15	111
44	M	3.8	0.1	L	3	100	16	66	3.7	101	17	72
45	F	4.4	0.1	L	2	105	19	154	4.3	105	15	199
46	M	4.9	3.2	R	3	112	19	73	5.0	104	15	84
47	M	13	85	L	2	156	42	306	13	138	31	158
48	F	5.2	0.4	L	2	110	16	11	5.2	106	20	83
49	F	3.6	0.4	R	4	105	23	145	3.6	105	18	72
50	M	9.7	11	L	3	128	25	101	9.8	132	33	141
51	M	5.5	5.3	R	4	115	21	86	5.4	116	22	137
52	M	9.7	4.1	B	3	138	34	67	9.5	140	47	66
53	M	9.7	69	L	3	140	49	174	9.5	136	33	176
54	M	8.8	60	L	4	137	31	115	8.8	139	32	217
55	M	6.7	29	L	3	123	27	248	6.7	125	25	113
56	M	5.0	0.6	R	1	118	23	122	5.0	95	15	48
57	M	5.8	22	L	2	111	24	181	6.0	114	18	51
58	M	11	28	L	3	146	30	232	11	153	40	293
59	M	5.0	1.1	R	1	116	21	125	5.2	114	21	118

A–I Perthes' group
A ID-number
B Sex (identical with respective control)
C Age at the time of examination (years)
D Months between diagnosis (in case of bilateral disease: diagnosis of second affected hip) and examination
E Affected hips
R right
L left
B bilateral
F Catterall group
G Standing height (cm)
H Weight (kg)
I IGF I plasma level (ng/mL)

K–N matched controls
K Age at the time of examination (years)
L Standing height (cm)
M Weight (kg)
N IGF I plasma level (ng/mL)

Table 2. IGF I plasma levels in children with Perthes' disease and matched controls. Mean *SEM*

	n	IGF I (ng/mL plasma)	*P*-value
All subjects			
Perthes'	59	125 *11*	0.049
Controls	59	139 *11*	
Within 2 years after diagnosis of Perthes' disease			
Perthes'	45	98 *7*	0.006
Controls	45	127 *11*	
More than 2 years after diagnosis of Perthes' disease			
Perthes'	14	211 *31*	0.5
Controls	14	175 *27*	

P-values are based on Wilcoxon's signed ranks test.

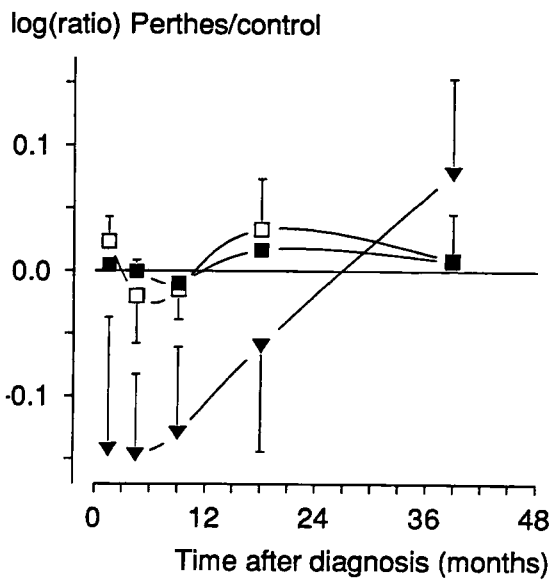


Figure 1. Relative values for IGF I plasma levels (triangles), standing height (filled squares) and weight (hollow squares) expressed as log(ratio[Perthes'/matched controls]), means ± SEM, for 59 children with Perthes' disease, plotted against time after diagnosis. 0–3 months: n 16, 3–6 months: n 5, 6–12 months: n 13, 12–24 months: n 11, >24 months (median 39 months): n 14.

hospital, previous nutritional habits could not be monitored. On clinical examination, however, no overt signs of malnutrition were noted in either group.

EDTA plasma samples were obtained from all children without prior fasting. The venepuncture was typically performed between 8 and 12 a.m.; blood samples were centrifuged at 2000 g for 5 min, and the plasma stored at –20 °C until further use. IGFs, unlike GH, have a long plasma half-life, resulting in plasma levels without major diurnal variation (Kaufmann et al. 1977, Hintz 1981).

Body mass and standing height were assessed with calibrated standard apparatus (SECA Inc., Germany) by one observer in the Perthes group and four observers in the control group. At the end of the study the preliminary control group was matched to the Perthes group for age and sex. The best matches for age were selected to form the definite control group of 48 boys and 11 girls with mean age 7 (3–14) years, age matched within ± 3.5 percent.

Total IGF I plasma levels in the Perthes and control groups were measured by radioimmunoassay (IRE Medgenix Inc., Fleurus, Belgium) after acid ethanol extraction (Daughaday et al. 1980). Cross-reactivity in this assay is below 0.02 percent for IGF II, and below 0.001 percent for insulin, GH, and EGF, according to the manufacturer's specifications. Results were expressed as ng IGF I/mL plasma.

Differences between group means were tested with the Wilcoxon signed ranks test, and *P*-values below 0.05 were considered significant.

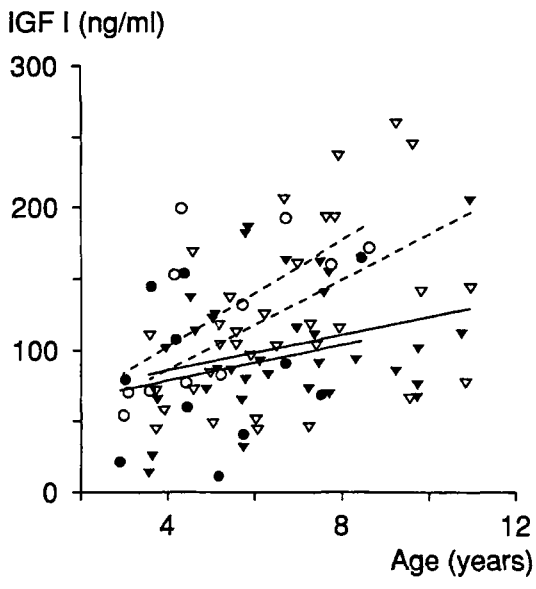
Results

IGF I plasma levels were lower in the Perthes than in the control group ($P < 0.05$, Table 2). This decrease was confined to the first two years of the disease ($P < 0.01$) and followed by a non-significant rebound (Figure 1).

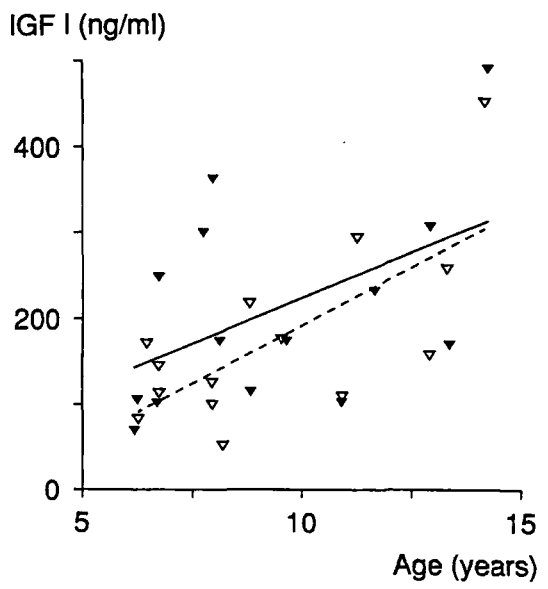
Linear regression analysis demonstrated a mitigated increase of IGF I plasma levels with advancing age in the Perthes group within the first 2 years of the disease, but not thereafter (Figure 2).

Children with Perthes' disease and matched controls did not differ in overall height and weight (Table 3), but there was a tendency towards growth arrest and impaired weight-gain during early-stage disease, followed by catch-up growth and relatively increased weight-gain.

No relation was found between femoral head involvement, and IGF I plasma concentrations or body weight (Table 4).



Values for 45 children with Perthes' disease within the first two years after diagnosis and their matched controls. Perthes' boys: $r = 0.28$, $P = 0.10$, control boys: $r = 0.41$, $P < 0.02$, Perthes' girls: $r = 0.22$, $P = 0.52$, control girls $r = 0.64$, $P < 0.05$. One outlyer (control ID-number 1) is not reproduced.



Values for 14 children with Perthes' disease more than two years after diagnosis, and their matched controls. Perthes' boys: $r = 0.49$, $P = 0.08$, control boys: $r = 0.70$, $P < 0.01$

Figure 2. IGF I plasma levels in children with Perthes' disease (filled symbols) and matched controls (open symbols). Triangles: boys, circles: girls. Linear regression lines: continous: Perthes group, dashed: control group; short lines: girls, long lines: boys.

Table 3. Age, standing height (cm) and weight (kg) of children with Perthes' disease and matched controls (n 59 per group). Mean (range) *SEM*

	At diagnosis of Perthes' disease	At determination of IGF I plasma levels		
	Age	Age	Height	Weight
Perthes'	6 (2–12)	7 (3–14)	121 *15*	25 *1*
Controls		7 (3–14)	120 *15*	24 *1*

Table 4. IGF I plasma levels (mean *SEM*) and body-weight in relation to radiographic findings in the hips of 59 children with Perthes' disease, grouped according to Catterall (1971)

Catterall group	n	IGF I (ng/mL plasma)	Weight (kg)
1	6	125 *15*	27 *3*
2	26	115 *19*	24 *2*
3	15	139 *23*	28 *2*
4	12	128 *22*	23 *2*

Discussion

Signs of disturbed skeletal maturation which reportedly accompany the femoral head necrosis in Perthes' disease (Burwell 1988) include retarded bone-age (Fisher 1972, Harrison et al. 1981, Kristmundsdottir et al. 1987) and disproportionate growth pattern with relatively short forearms and feet (Burwell et al. 1978, Hall et al. 1988). Our data confirm the results of Exner and Schreiber (1986) who reported an impaired growth-velocity during early-stage disease, followed by catch-up growth. Some observers have found that children with Perthes' disease have a short stature for their age (Wynne-Davies and Gormley 1978, Hall et al. 1988); others have failed to confirm this (Laron et al. 1973).

The metabolism of growth-related hormones such as GH, thyroid hormones and adrenal steroids in Perthes' disease seems to be normal (Chapman 1956, Fisher 1972, Laron et al. 1973, Tanaka et al. 1984), but no agreement exists on the somatomedins. Tanaka et al. (1984), using radioreceptor-assay, found low

plasma levels of somatomedin A in Perthes' disease. It has meanwhile been demonstrated that Sm-A and Sm-C/IGF I are identical (Baxter and Martin 1989). Work based on bio-assays, in contrast, yielded a tendency towards elevated Sm-levels in affected children (Burwell et al. 1986, Rayner et al. 1986). Cartilage bio-assays, however, are not specific for IGF I but may be influenced by various other peptides (Prins et al. 1982a, b). Normal IGF I-values in Perthes' disease were reported by Kitsugi et al. (1989), who employed a radioimmunoassay.

All of the above-cited previous results on IGFs were obtained on the basis of unextracted serum or plasma, raising questions regarding their validity. In humans, the major part of circulating IGF I is bound to a protein-complex (Binoux et al. 1986). In the bound form, the bio-activity of IGF I is reduced (Baxter and Martin 1989) and access to membrane-receptor- and immuno-binding-sites is inhibited (Daughaday et al. 1980). The results of RIAs, radio-receptor-assays, and bio-assays performed without prior extraction may thus be adversely influenced.

Acid ethanol extraction, as used by us, has been demonstrated to reduce interference with IGF binding proteins in an IGF-RIA (Daughaday et al. 1980). Even with this step, however, such interference still cannot be totally excluded.

Low levels of circulating immuno-reactive IGF I in Perthes' disease and failure of IGF I to increase normally during the prepubertal years, as demonstrated in the present study, may reflect an impaired synthesis and/or release of this growth factor in affected children relative to their chronological age. In view of reportedly normal GH levels, a possible cause of these phenomena could be a decreased responsiveness to GH of the target-cells, notably growth-plate chondrocytes and hepatocytes. Possible alterations in the plasma concentration or affinity for IGF I of the IGF binding proteins also have to be considered. Malnutrition can lead to low IGF I levels (Hintz 1981), and some observers have found a higher incidence of Perthes' disease among children of low-income families (Wynne-Davies and Gormley 1978). Our Perthes' patients, however, were neither shorter nor did they weigh less than the controls. The proportion of patients with medical insurance coverage provided by a public social institution was not higher than in the control group.

As a normal skeletal development is, among other factors, dependent on IGF I, our data correspond well with the reportedly impaired bone-maturation in children with Morbus Perthes. Like the decrease in plasma-IGF I, the skeletal retardation is already present at the time of diagnosis (Kristmundsdottir et al. 1987), suggesting a latent period between the onset of systemic alterations and the femoral head necrosis. The time-course of plasma IGF I-levels, standing height, and body mass seems to indicate that these parameters are altered in a parallel fashion at least during the first 2 years after the diagnosis. The trend back to normal values observed towards the end of the disease episode furthermore suggests that these alterations are of temporary nature. The unknown space of time between development of the femoral head necrosis and diagnosis, however, should be borne in mind when interpreting these data.

The relation between systemic and local changes in Perthes' disease is at present not clear. Harrison and Burwell (1981) suggested that the impaired skeletal maturation in Perthes' disease could leave the proximal femur less competent to bear weight, resulting in stress-fractures with subsequent vascular insufficiency. No correlation, however, was found between degree of femoral head involvement, according to Catterall (1971), and IGF I plasma levels (Tanaka et al. 1984, Kitsugi et al. 1989, own data), body mass (own data), or degree of bone age retardation (Kristmundsdottir et al. 1987).

Low levels of plasma IGF I, retarded bone age, and infantile body proportions may be symptoms of a single developmental disorder, and children who display these signs may be at an elevated risk to develop Perthes' disease. The factors, however, which eventually determine the extent of the femoral head necrosis, have not been identified.

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