Effect of Pubertal Stage and Recent Blood Glucose Control on Plasma Somatomedin C in Children with Insulin-dependent Diabetes Mellitus

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SUMMARY

One hundred and fifty-five children with insulin-dependent diabetes mellitus were studied to determine the relationship of plasma somatomedin C concentrations to pubertal stage, recent blood glucose control, duration of diabetes, and daily insulin dosage. Plasma somatomedin C, as measured by radioimmunoassay, increased with age and the progression of puberty in both boys and girls, but was not different from those of normal children aged 5–18 yr. In diabetic children under 5 yr of age, plasma somatomedin was significantly lower than in normal children. These results are different from those reported previously in diabetic children, in which somatomedin activity was measured using a pig cartilage bioassay.

In boys, puberty was accompanied by an increase in hemoglobin A_{1c} from 11.6 to 14.1% despite a 36–44% increase in total daily insulin dosage adjusted for body weight. In girls, puberty was also associated with an increase in hemoglobin A_{1c} (12.1–15.1%) without a change in weight-adjusted insulin dose. Although there was no apparent correlation between hemoglobin A_{1c} and somatomedin C in the group as a whole ($r = -0.078$), multiple regression analyses demonstrated a significant negative correlation between these two variables ($P < 0.001$) when considered independently of age, sex, pubertal stage, duration of diabetes, and insulin dose. Pubertal status had a significant ($P < 0.01$) effect on plasma somatomedin C; plasma somatomedin C was also significantly positively correlated with the duration of diabetes ($P < 0.01$) but not with weight adjusted insulin dose. SMC concentration was not correlated with age when this variable was considered independently of puberty ($P = 0.26$). The data indicate that puberty and metabolic control both have significant yet independent effects on somatomedin C in children with insulin-dependent diabetes. In addition, the increased somatomedin C associated with puberty may contribute to the accelerated development of diabetic complications seen at this time, while the lowering of somatomedin C associated with worsening metabolic control may adversely affect linear growth. **DIABETES *30*:868–872, October 1981.**

Although the Mauriac syndrome [hepatomegaly, obesity, and short stature in children with poorly controlled insulin-dependent diabetes (IDD)] is generally thought to be the result of extremely poor metabolic control,[1] the effect of the varying levels of blood glucose regulation more typically found in children with IDD on somatic growth is less clear. Although some children treated with a regimen of regular insulin twice a day have been reported to have decreased final adult heights,[2] more recent studies suggest that most children with "fair" (as defined by the respective authors) or better metabolic control grow normally.[3,4]

The somatomedins are a group of growth hormone-dependent, mitogenic polypeptides that mediate certain actions of growth hormone on cartilage.[5] Because of their growth promoting effects, the levels of somatomedins in diabetes are relevant to the study of the effect of blood glucose control on growth in children.

Previous studies in patients with diabetes have reported low, normal, and elevated somatomedin levels using a wide variety of bioassays and radioreceptor assays for somatomedin activity. The majority of studies in which somatomedin was measured with a cartilage bioassay have shown decreased somatomedin activity,[6–8] but Cohen et al.[9] reported elevated somatomedin activity in adult diabetics, while Nash[10] found normal somatomedin activity in diabetic children who were being treated with insulin. Franklin et al.[11] also found no changes in somatomedin activity with a rat

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adipocyte assay when adult diabetics were studied before and 3–4 mo after insulin therapy.

In animal models, untreated diabetes induced either by streptozotocin[12–14] or partial pancreatectomy[15] results in decreased somatomedin in both bioassays[12,15] and radioreceptor assays.[13,14] Treatment with insulin results in normalization of somatomedin levels, even though euglycemia is not always attained.[12,15]

There appear to be at least three reasons for these discordant results. First, there are several different polypeptides that are considered to be somatomedins.[5,16] These peptides differ in their metabolic effects and in the extent to which they are active in different somatomedin assays.[17,18] One class of somatomedin is exemplified by somatomedin C (SMC) and insulin-like growth factor-I (IGF-I). The serum levels of these somatomedins are more closely related to growth hormone status than are those of the other class of somatomedins exemplified by insulin-like growth factor-II (IGF-II), which is more insulin-like in its actions. The extent to which the different bioassays that have been used to study somatomedins in diabetes measure different somatomedin polypeptides is not yet completely established. The rat cartilage bioassay preferentially detects SMC and IGF-I, while IGF-II is more potent in the rat adipocyte assay. In the human placental radioreceptor assay, IGF-II is about one-third as potent as SMC and IGF-I.[17,18]

Second, there are inhibitors in diabetic serum which blunt somatomedin action on cartilage.[19]

Third is the difficulty in defining and quantifying the degree of metabolic control in diabetic children. The discovery that diabetics have increased amounts of glycosylated hemoglobins and the development of rapid and convenient assays for these hemoglobins have made it possible to evaluate long-term diabetic control in a more quantitative fashion than was previously available from random blood or urine glucose measurements.[20]

We report studies of SMC as measured with a specific radioimmunoassay[21] in 155 children with IDD. In these studies, diabetic control was evaluated by measurements of hemoglobin A_{1c} (HbA_{1c}), and the relationship of SMC and diabetic control was correlated with additional factors, such as pubertal status, age, sex, duration of IDD, and insulin dose, which might affect either plasma SMC[22] or metabolic control.[23]

MATERIALS AND METHODS

The study population consisted of 136 children (67 boys and 69 girls) with established (greater than 1 yr in duration) IDD and 19 children (12 boys and 7 girls) who had been diabetic for 1–9 mo. They were studied as part of the Diabetes Registry program of the Washington University Diabetes Research and Training Center. The patients' height, weight, and pubertal development[24,25] were recorded at each clinic visit. The children ranged in age from 1.75 to 20 yr, and except for four children who were receiving thyroid hormone replacement, they were taking no medication except insulin. No patients with intestinal malabsorption or elevated serum creatinine were studied. All were within ±2 SD of the mean height for age. None of the children with established diabetes had been hospitalized for diabetic ketoacidosis in the 3 mo before study.

Blood for determination of HbA_{1c} by liquid chromatography[26] was drawn into EDTA-containing tubes and kept at 4°C until separated. Packed red blood cells were maintained at 4°C until assayed within 4 days of collection. Under these conditions, the mean difference of hemoglobin A_{1c} between saline washed red cells assayed immediately after sampling and packed cells stored for 4 days is less than 5% of the total value. Normal values for children are 5.9 ± 1.3 (mean ± SD) and do not change with puberty. The plasma obtained from these specimens was stored frozen (−20°C) for up to 10 mo until assayed for SMC by the non-equilibrium radioimmunoassay of Furlanetto et al.[21] These samples were collected at each clinic visit, the majority of which occurred in the morning after breakfast.

TABLE 1
Characteristics of children with established insulin-dependent diabetes

Pubertal stage	Sex (number)	Age* (yr)	Duration* (yr)	Insulin dose* (U/kg/day)
1	M (27)	7.9 ± 0.5	4.0 ± 0.5	0.75 ± 0.03†
	F (19)	7.6 ± 0.6	3.2 ± 0.3	1.03 ± 0.09
2	M (10)	12.3 ± 0.5†	4.2 ± 0.8	0.83 ± 0.09
	F (5)	10.6 ± 0.02	4.0 ± 1.2	1.03 ± 0.14
3	M (12)	13.9 ± 0.5	3.9 ± 0.7	1.02 ± 0.14
	F (21)	13.1 ± 0.2	5.2 ± 0.7	1.01 ± 0.06
4	M (8)	15.4 ± 0.3‡	7.4 ± 1.3	1.08 ± 0.14
	F (21)	13.5 ± 0.8	5.4 ± 0.8	1.07 ± 0.08
5	M (23)	16.7 ± 0.3	6.9 ± 0.8	0.93 ± 0.09
	F (27)	16.4 ± 0.3	7.3 ± 0.7	0.86 ± 0.11

* mean ± SEM.
† Difference between boys and girls significant ($P < 0.01$).
‡ Difference between boys and girls significant ($P < 0.05$).

The rabbit antibody to SMC used in these studies was obtained from the National Pituitary Agency, Baltimore, Maryland and used at a final dilution of 1:20,000. [^{125}I]Somatomedin C purified by affinity chromatography[17] was the generous gift of Drs. J. J. Van Wyk and L. E. Underwood of the University of North Carolina. A commercial pool of serum from normal adults (lot #1778-5 Ortho Diagnostics, Raritan, New Jersey) was used as a standard at an assigned potency of 1.66 U/ml. When the serum standard was defined in this fashion, the mean SMC of a population of 220 normal adults was 0.9 U/ml with 95% of the population falling in a range between 0.4–2.0 U/ml. Plasma SMC values for normal children (mean ± SD) with this standard are as follows: 0–5 yr 0.79 ± 0.44 U/ml (N = 33), 5–8 yr 1.05 ± 0.54 U/ml (N = 16) and 8–18 yr 1.78 ± 1.02 U/ml (N = 21). (These determinations were performed in Dr. Van Wyk's laboratory.)

Statistical significance between groups of children was determined using the unpaired Student's *t* test. A multiple regression model was used to relate SMC to each of the following variables: HbA_{1c}, Tanner stage, sex, duration of IDD, and daily weight adjusted insulin dose.

RESULTS

The characteristics of the children with established IDD and those of the children with diabetes for less than 1 yr are shown in Tables 1 and 2. Among the children with established IDD, the prepubertal boys were receiving an average of 0.28 U/kg less insulin than the prepubertal girls ($P < 0.01$). This difference in insulin dose between prepubertal boys and girls was not seen in the smaller group of children

TABLE 2
Characteristics of children with recent onset of IDD

Pubertal stage	Sex	Number	Age* (yr)	Duration* (yr)	Insulin Dose* (U/kg/day)	HbA_{1c}* (%)	SM-C* (U/ml)
1	M	6	6.6 ± 0.9	0.29 ± 0.08	0.48 ± 0.09†	10.8 ± 1.5	1.63 ± 0.55
	F	6	6.3 ± 1.1	0.25 ± 0.12	0.57 ± 0.08‡	10.9 ± 1.2	1.25 ± 0.23
2	M	1	12.0	0.04	0.26	17.3	1.60
3	M	1	16.0	0.12	0.69	9.2	3.02
5	M	4	16.0 ± 0.8	0.12 ± 0.04	0.41 ± 0.13§	10.3 ± 1.7	2.90 ± 0.85
	F	1	15.25	0.80	0.33	10.6	3.28

* mean ± SEM.
† Significantly ($P < 0.01$) less than prepubertal boys with established IDD.
‡ Significantly less than ($P < 0.001$) prepubertal girls with established IDD.
§ $P < 0.05$ compared with P-5 boys with established IDD.

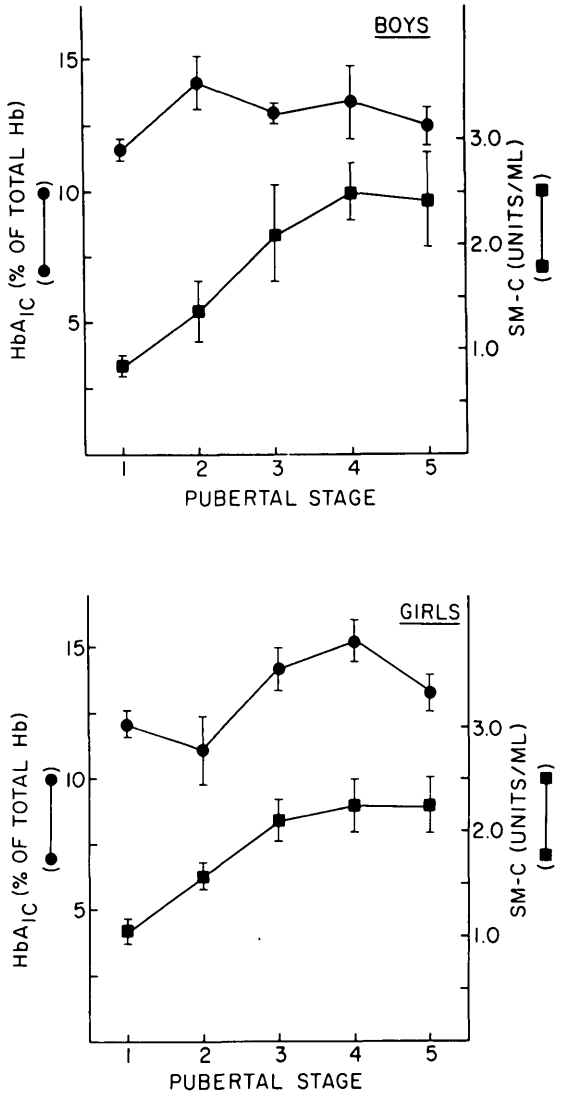
with recent onset of diabetes. The children with IDD for less than 1 yr were receiving 40–50% less insulin than those with established diabetes ($P < 0.01$ for prepubertal boys; $P < 0.001$ for prepubertal girls; $P < 0.05$ for boys in late puberty). In boys with established IDD, the insulin dose increased during the first to fourth stages of puberty while in girls there were no changes in daily insulin.

The relationship of pubertal stage to HbA_{1c} and SMC is shown in Figure 1. In boys, HbA_{1c} levels were significantly ($P < 0.05$) greater in early puberty (P-2 and P-3) than prior to puberty. In girls, very early puberty had no adverse affect on HbA_{1c}, but mid to late puberty was accompanied by significant worsening of diabetic control, as indicated by elevated HbA_{1c}. Where it was possible to make statistical comparisons (P-1 boys and girls, P-5 boys), there was no significant difference in either HbA_{1c} or SMC between children with recently diagnosed and established diabetes.

When the children were considered as a group, SMC was significantly correlated with age ($r = 0.45$, $P < 0.001$) and poorly correlated with HbA_{1c} ($r = -0.078$, $P < 0.2$). However, since age and pubertal status are related, and it appears that puberty affects HbA_{1c} (Figure 1), a multiple regression model was used to relate each of these variables to SMC. The results of this analysis are shown in Table 3. With this method of analysis, there was a significant negative correlation between HbA_{1c} and SMC. Pubertal status was also significantly related to SMC, as was duration of IDD. Age-, sex-, and weight-adjusted insulin dose were not significantly correlated with SMC.

Although there is no significant difference between children with IDD and normal children over the age of 5 yr, when the data on SMC are grouped by age without regard to gender or stage of pubertal development, plasma SMC was significantly lower in the seven samples from four children less than 5 yr old (0.40 ± 0.09 U/ml, mean ± SEM, N = 7 vs. 0.79 ± 0.08, N = 33, $P < 0.01$).

FIGURE 1. Relationship of levels of HbA_{1c} and somatomedin C to pubertal development in children with IDD for over 1 year. Each point is shown as mean ± SEM. (A) Boys. The difference in HbA_{1c} between P-1 boys and P-2 and P-3 boys is significant ($P < 0.05$). Somatomedin C is significantly lower ($P < 0.02$, $P < 0.01$, and $P < 0.001$) in P-1 boys as compared with P-3, P-4, and P-5 boys, respectively. (B) Girls. HbA_{1c} is significantly lower in P-1 girls than in P-3, P-4, or P-5 girls ($P < 0.05$, $P < 0.001$, and $P < 0.02$). Somatomedin C is significantly lower in P-1 girls compared with girls in other stages of puberty (P-1 vs. P-2, $P < 0.01$, $P < 0.001$ for the others). Somatomedin C is significantly ($P < 0.05$) lower in girls in early puberty, P-2, as compared with those in later puberty.



DISCUSSION

Because of the independent and concomitant increase of SMC and HbA_{1c} during puberty, the strong negative correlation of SMC with HbA_{1c} was not readily apparent. However, this correlation becomes evident following multiple regression analysis and confirms the observations that Winter et al.[8] made using the rat cartilage bioassay. These results suggest that the decrease in somatomedin bioactivity observed by these and other authors reflects an actual de-

TABLE 3
Multiple regression analysis of the factors affecting SMC in children with IDD

Variable	F	df	P
HbA_{1c} (%)	13.34	1157	<0.001
Pubertal stage	3.45	4157	<0.01
Duration (yr)	6.45	1157	<0.01
Insulin dose (U/kg/day)	3.47	1157	0.06

crease in SMC peptide levels as well as the effects of any inhibitors which may be present. The normal somatomedin levels reported by Nash[10] may reflect the fact that eight of the 10 patients studied were pubertal and that while their somatomedins were within normal limits for adults, they may actually have been low for pubertal children. Cohen et al.[9] used chick embryo fibroblasts as their test system. IGF-I (and presumably SMC) and IGF-II both bind equally well to these cells[18] and it seems likely that this assay is measuring both of these peptides. Since IGF-II is more potent in the rat adipocyte assay for somatomedin A and serum IGF-II concentrations are considerably higher than those of SMC (IGF-I), the lack of effect of insulin treatment on somatomedin reported by Franklin et al.[11] may be due to a lack of effect on IGF-II.

In normal children, SMC varies with age and sex when measured either with the specific radioimmunoassay or with the less specific human placental radioreceptor assay.[21,22,27] Underwood et al.[22] reported that immunoassayable SMC levels reached a peak in 13-yr-old boys and girls. Although these children were not classified as to their pubertal status, the data of Marshall and Tanner[24,25] suggest that most of the girls and one-half of the boys would be in pubertal stage P-4. Markedly increased somatomedin in the cartilage bioassay[28] and in the somatomedin A radioimmunoassay have also been noted[29] in pubertal children (note that IGF-I is 10 times more potent than somatomedin A in this assay[30]). The increase in immunoassayable SMC in children with IDD during puberty reported here thus appears to be similar to the changes seen in normal children. It is interesting to note that SMC concentrations are maximal at approximately the same stage of puberty where growth velocity is maximal.[25]

The question as to whether chronologic age or stage of puberty is the more important factor in determining plasma SMC has not yet been answered for normal children.[31] Our data suggest that when age and pubertal status are analyzed separately, the hormonal changes of puberty are a more important determinant of SMC than is age.

Puberty is accompanied by multiple endocrine and metabolic changes.[32] These changes are reflected not only in increased SMC but in worsening of metabolic control in diabetic teenagers. This worsening of control appears to be due in part to relative insulin resistance since both improved control and decreased insulin requirements are found in older teenagers.[23] A recent study[33] indicates that 66% of normal adolescents have defective growth hormone suppression after an oral glucose load. This paradoxical growth hormone response may explain the increase in SMC levels while serving as a contributing factor to the apparent insulin resistance seen in diabetic teenagers.

The increased levels of the growth factor SMC in puberty is of special interest in diabetes. Clinically, the incidence of long-term complications, such as retinopathy or nephropathy, is less in prepubertal diabetics than in children with the same duration of disease but with onset of diabetes after onset of puberty. It has been reported that duration of diabetes after the onset of puberty, but not the total duration of diabetes, was significantly associated with quadriceps muscle capillary basement membrane thickening.[34] The apparent acceleration of microvascular disease in diabetic children after the onset of puberty may be due, in part, to the combination of the metabolic derangements resulting from a deterioration of diabetic control and an increase of plasma SMC, a potent mitogen.

The decreased plasma SMC associated with deteriorating diabetic control in adolescence might also result in a blunting of the adolescent growth spurt and lead to a decrease in ultimate adult height. This effect would be particularly important in children with longstanding IDD who are at the greatest risk of growth failure.[2-4] Both of these factors would seem to justify intensive efforts to maintain good metabolic control during puberty.

Lastly, studies which attempt to relate somatomedin levels to metabolic control in diabetic children need to be planned so that normal pubertal changes in somatomedins are not ascribed to differences in diabetic management. This caveat is especially relevant to long-term studies employing intensive efforts at blood glucose control in children who will be entering into or progressing through puberty during the study.

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