Effect of Glycemic Control on Serum Insulin-like Growth Factors in Diabetes Mellitus

THOMAS J. MERIMEE, DONALD F. GARDNER, J. ZAPF, AND E. R. FROESCH

SUMMARY

We investigated the effect of improving glycemic control on serum concentrations of insulin-like growth factors I and II (IGF-I and IGF-II). In 22 adults followed during an intensive home glucose monitoring program for 6 mo, no effect of improving control was seen on either IGF-I or IGF-II. Similar results were obtained in young diabetic children <10 yr of age and in diabetic adolescents with detectable puberty before entering the study. In older diabetic children without evidence of puberty before treatment (Tanner prepubertal stage 1), initial IGF-I concentrations were low, but increased during establishment of glycemic control. Puberty developed during therapy in this latter group.

Our data do not support a "global" effect of glycemic control on serum IGF-I in diabetic patients. Increases of IGF-I with better glycemic control appear most likely to occur when the metabolic consequences of diabetes have suppressed normal pubertal increases of IGF-I. IGF-II concentrations were unaffected by glycemic control in all subjects. DIABETES *33*:790–793, August 1984.

Many actions previously attributed to growth hormone are now thought to be mediated by secondary growth factors.[1-4] Two such factors have been isolated in man, their amino acid structure determined, and specific immunoassays constructed for each.[5-8] Insulin-like growth factor I (IGF-I), which is identical to somatomedin C, and IGF-II, are both homologous to proinsulin[6,7] and both are bound in serum by one or more proteins.[9-12] We previously presented evidence for an association between increased serum concentrations of IGF-I and severe diabetic retinopathy.[13] It was our impression that glycemic control did not relate to the concentration in serum of either factor I or II. The current studies were undertaken to examine this problem more adequately.

From the Department of Medicine, Endocrinology Division, University of Florida, Gainesville, Florida (T.J.M.); the Methodist Hospital, Houston, Texas (D.F.G.); and the Department of Internal Medicine, University Hospital, Zurich, Switzerland (J.Z., E.R.F.).

Address reprint requests to Dr. T. J. Merimee, Department of Medicine, Endocrinology Division, University of Florida, Gainesville, Florida 32610.

Received for publication 14 September 1983 and in revised form 18 January 1984.

MATERIALS AND METHODS

Subjects. Arrangements were made for 22 diabetic adults requiring insulin to be followed during an intensive home glucose monitoring program. None of these subjects had rapidly accelerating retinopathy. Fifteen of these 22 subjects, who had been managed on insulin for at least 5 yr, had serum concentrations of IGF-I and IGF-II before treatment that did not differ significantly from the mean IGF-I and -II concentrations recorded in normal subjects; i.e., IGF-I ranged from 198 to 242 ng/ml and IGF-II concentration ranged from 510 to 690 ng/ml in these 15 patients. Five adult diabetic patients also managed on insulin had low IGF-I concentrations by immunoassay (< 100 ng/ml) and 3 of these 5 had low IGF-II concentrations (< 300 ng/ml). Two patients had IGF-I values > 500 ng/ml.* These "low and high" values had been noted on two consecutive determinations. In all groups, the ages of the subjects varied from 29 to 58 yr. The technique used for achieving better glycemic control in these patients is summarized elsewhere in greater detail.[14] It consisted of a minimum of two injections of insulin daily with supplementary insulin being given on the basis of monitoring of blood glucose four times a day. Glucose was monitored initially daily; later, twice weekly.

Serum and whole blood samples were collected at the beginning of the program and at 3- and 6-mo intervals during the home glucose monitoring period. Serum samples were analyzed for IGF-I, IGF-II, and glycosylated serum protein levels. Whole blood samples were used for determining hemoglobin A_{1c} (HbA_{1c}).

*There is considerable overlap of IGF-I and IGF-II values between control and diabetic subjects. Only subjects with rapidly accelerating retinopathy have gross elevations of IGF-I. Data on these subjects are not included in the current report (see ref. 13).

TABLE 1
Effect of glycemic control on mean IGF-I and IGF-II in adult subjects with normal initial values of IGF-I

	Initial	3 Mo	6 Mo
IGF-I (ng/ml)	204 ± 20	229 ± 19	230 ± 10
IGF-II (ng/ml)	669 ± 59	621 ± 55	566 ± 69
HbA_{1c} (%)	14.8 ± 0.95	10.7 ± 0.82*	10.3 ± 0.8*
Glycosylated serum protein (nM)	1.00 ± 0.17	—	0.64 ± 0.06*

Data are given as mean ± SEM.
*Indicates values differ from the control or 0 time with a P value of at least 0.01.

Five children between the ages of 9 and 10 yr, and 7 between the ages of 13 and 15 yr, were placed on pump treatment and similar studies conducted. Samples in children were collected at the beginning of the study and at 2-mo intervals for 6 mo.

Biochemical techniques/biochemical procedures. One-tenth milliliter of each serum sample was first extracted by the column chromatography method of Zapf.[8] IGF-I and IGF-II assays were then carried out at 4°C in PBS-0.2% HSA buffer, pH 7.4, using 0.1 ml of the serum extract.[8] Each serum extract was measured in duplicate at two different dilutions (undiluted and diluted 1:2 for IGF-I, dilutions of 1:2 and 1:4 for the IGF-II assay). Eight to 10 internal controls were run with each assay. Cross-reactivity of IGF-II in the IGF-I assay was 1%; cross-reactivity of IGF-I in the IGF-II assay was 10%. Values in this article have been corrected for the cross-reactivity. Details for determining measurements of assay variability and cross-reactivity are discussed in detail in a previous publication.[8]

We measured glycosylated HbA_{1c} and glycemic control, using the thiobarbituric acid assay for determining glycosylation. In this assay, hydroxymethylfurfural (HMF) is generated from released carbohydrate and quantitated colorimetrically with thiobarbituric acid, using pure HMF for the standard curve.[15] We converted OD values for glycosylated hemoglobin by the thiobarbituric assay to percent HbA_{1c} by use of a linear equation obtained by methods described elsewhere in detail.[16] The upper limit of normal for 35 controls for glycosylated HbA_{1c} was 10.4% (mean ± 2 SD). Values for glycosylated serum protein, determined also by a thiobarbituric method,[17,18] are given as nanomoles of HMF.

RESULTS

During the 6-mo study period in adult subjects, HbA_{1c} decreased significantly as did glycosylated serum protein. The initial HbA_{1c} in the 15 adult subjects was 14.8 ± 0.95% decreasing to 10.7 ± 0.82% and 10.3 ± 0.80% at 3 and 6 mo, respectively. Glycosylated serum protein decreased from a mean concentration of 1.00 ± 0.17 nM to 0.64 ± 0.06 nM at 6 mo. The 3-mo values were not obtained due to a technical error in the processing of these samples. The normal concentration and standard deviation for HbA_{1c} in our laboratory is 8.1 ± 1.2% and for glycosylated serum protein, 0.32 ± 0.13 nM. During the time period that HbA_{1c} and glycosylated serum protein were decreasing in adults, no significant changes occurred in IGF-I or IGF-II. These mean data are summarized in Table 1.

Since there is a considerable variation in the basal concentrations of IGF-I and IGF-II in man, results were also examined by calculating percent changes from basal for IGF-I and IGF-II (see Figure 1). IGF-I concentrations in serum at 3 and 6 mo were 113 ± 5% and 105 ± 16%, respectively, of the pretreatment value (P = NS for both comparisons). IGF-II concentrations were 99 ± 4.6% and 98 ± 7.1% of the basal concentration, respectively. Linear regression studies revealed no correlation of IGF-I and IGF-II to HbA_{1c} (for IGF-I–HbA_{1c}, r = 0.03024).

Five subjects, in addition to the 15 just cited, were selected because of low IGF-I concentrations in serum (see MATERIALS AND METHODS). Results of better glycemic control in these subjects are shown in Table 2. Only 1 of the 5 subjects had a substantial rise of IGF-I at 3 mo that was sustained at the 6-mo review period. This subject had the poorest overall control of glucose both at the beginning and at the end of the treatment period.

The effect of glycemic control on IGF-I in children and adolescents is shown in Figure 2. In younger children, ages 8–9.5 yr without evidence of puberty, better glycemic control had no effect on IGF-I concentrations in serum. In older children with evidence of puberty before treatment (first panel of B, Figure 2), no consistent change of IGF-I concentration occurred during treatment. In 3 older children (ages 15 yr) without evidence of puberty before treatment, initial IGF-I concentrations in serum were low and increased dramatically during establishment of glycemic control (second panel of B, Figure 2). IGF-II changes in all subjects were variable, with no discernible pattern.

DISCUSSION

An influence of growth hormone on the course of diabetic retinopathy has long been suspected.[19–26] However, since most major actions of growth hormone are now thought to

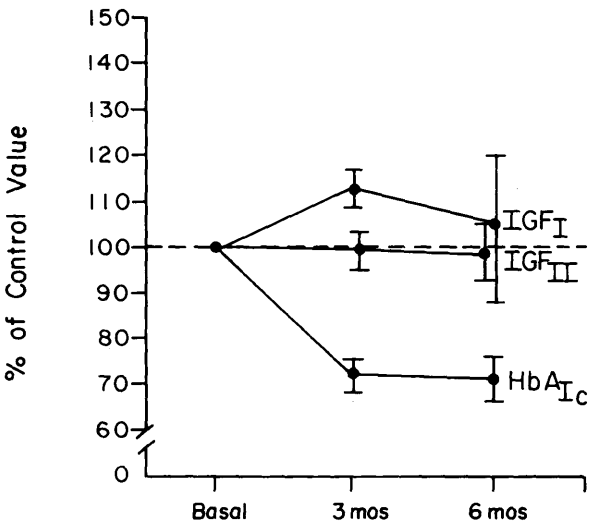

FIGURE 1. IGF-I, IGF-II, and HbA_{1c} are plotted as percent of the pretreatment concentration in serum. Points are means ± SEM. Only HbA_{1c} values differ from control, with P < 0.01.

TABLE 2
Effect of glycemic control on IGF-I and IGF-II in adult subjects with decreased initial values of IGF-I

	IGF-I (ng/ml)			IGF-II (ng/ml)			HbA_{1c}		
Time (mo)	0	3	6	0	3	6	0	3	6
Subject									
1	95	141	40	290	304	224	12.8	11	9.5
2	88	74	50	363	350	405	13.0	9	10.5
3*	109	234	221	704	752	893	18.3	13.9	14.6
4	66	100	106	393	386	357	15.7	7.9	6.9
5	96	104	—	718	685	—	16.0	8.4	8.0

*Only 1 of 5 subjects had a substantial rise of IGF-I at 3 mo that was sustained at 6 mo. This subject had the poorest overall control of glucose both at the beginning and end of the treatment period. Mean normal concentration of IGF-I by the extraction procedure used is 220 ± 17 ng/ml and mean normal concentration for IGF-II is 605 ± 36 ng/ml.

be mediated by secondary growth factors, it becomes important to characterize these factors in the diabetic state and to characterize the conditions that affect their fluctuation. We have shown in a previous article that gross elevation of IGF-I concentrations in serum characterizes subjects with rapid deterioration of retinal function.[13] A clear-cut effect of glycemic control on IGF values, however, was not evident to us.

Measurements of IGF or somatomedins in diabetic patients have been previously reported as low, normal, elevated, and related or nonrelated to glycemic control.[27-33] In retrospect, there are multiple reasons for such discrepancies that relate, in part, to the means of classifying diabetic subjects, the methods of measuring IGF and its inhibitors, as well as to problems of selection of patients and judgment of control. In the current study, we examined IGF-I and IGF-II before and after what we deemed sufficient time for adjustment of IGF to control; glycemic control was judged by measurements of glycemic status, which, although not infallible, theoretically give a superior assessment of the mean glycemic status of the patient.

In 15 adults with insulin-dependent diabetes and normal IGF values, we found no relationship between glycemic control and IGF-I and IGF-II concentrations in serum (Figure 1, Table 1). In 5 adult subjects with low IGF-I values (Table 2), we likewise found no effect of glycemic control on altering the serum concentrations of IGF-I or IGF-II. The same situation was noted in two adult patients with grossly elevated levels of IGF-I before treatment (not shown in results). It is possible that had control been poorer to begin with, the concentrations of IGF might have been lower and improved with control.

In a previous study, we noted IGF-II concentrations tended to be lower than normal in patients with type II diabetes even when they were receiving insulin therapy. We still have no explanation for this phenomenon nor its significance. Clearly, better glycemic control had no detectable effect on IGF-II.

In children, age and stage of puberty appear to be major factors in determining whether improvement of glycemic control results in an increase of IGF-I. In children between the ages of 8 and 10 yr without puberty, the establishment of better glycemic control was not associated with an increase of IGF-I. In 3 subjects age 15 yr with no evidence of puberty, low IGF-I concentrations noted initially increased during the improvement of glycemic control. We believe it important to emphasize that early signs of puberty were detected in these 3 while in the program, but not, as indicated, at the beginning of the study. In 4 subjects of the 12–15-yr age group with evidence of puberty before the establishment of glycemic control, IGF-I was normal initially. IGF-I increased modestly in 2 of these 4 and remained unchanged in the other 2. In subjects of all age groups, IGF-II values were normal initially and remained so during the home glucose monitoring program.

We would interpret the results in adults to indicate there is no consistent effect of glycemic control on the serum concentration of either IGF-I or IGF-II. In children, an explanation we favor is that in some, the metabolic abnormalities of diabetes inhibit the development of puberty and concomitantly inhibit the normal pubertal rise of IGF-I. Theoretically, this effect on IGF-I could be mediated by allowing a normal increase of GH secretion to occur at puberty and/or by preventing unresponsiveness to GH by unknown mechanisms. It should be pointed out that a similar effect of chronic disease on pubertal development is recorded in other conditions and is not specific to diabetes. In children with severe anoxic heart disease, for example, both puberty and growth are severely retarded, and correction of the defect causing anoxia usually results in the prompt onset of puberty.

In summary, our data would not support a "global" effect of glycemic control on serum concentrations of IGF. Rather,

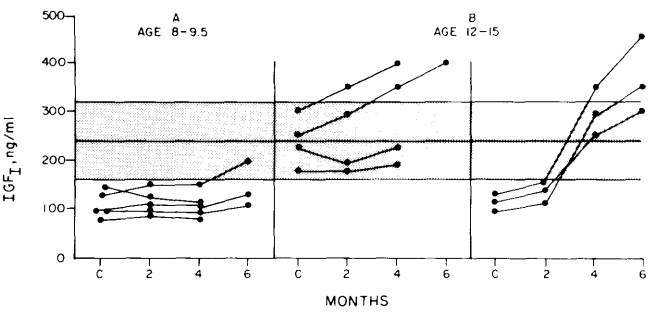

FIGURE 2. IGF-I concentrations in serum before and during treatment are shown for 12 children and adolescents. Shaded area indicates mean ± SD for adult subjects. An increase in IGF-I after better glycemic control in the 13–15-yr age group occurred only when the initial IGF-I was depressed. In the 3 subjects with significant increases of IGF-I (B, panel 2), signs of puberty were absent before establishing glycemic control, but were noted after start of therapy.

they indicate that only in selected subjects of a given age and stage of pubertal development can results be predicted. The initial depression of IGF-I in some subjects with diabetes is probably not due to diabetes per se, but is a general phenomenon common to many chronic diseases.

ACKNOWLEDGMENTS

This work was supported in part by a grant (AM-18130) from the National Institute of Arthritis, Metabolism, and Digestive Diseases, NIH; by grants from the Florida Citrus Commission and the General Clinical Research Center Program of the Division of Research Resources, NIH (RR-00082); by a grant (3.167.0.81) from the Swiss National Science Foundation; and by a contract with the Department of Health and Rehabilitative Services of the State of Florida for the University of Florida Diabetes Research Education and Treatment Center.

REFERENCES

[1] Hall, K., Raben, M. S., Salmon, W. D., Van den, J. L., and Van Wyk, J. J.: Somatomedin: a proposed designation the "sulfation factor." Nature 1972; 235:107.

[2] Schoenle, E., Zapf, J., Humbel, E. R., and Froesch, E. R.: Insulin-like growth factor I stimulates growth in hypophysectomized rats. Nature 1982; 296:253–53.

[3] Salmon, W. D., Jr., and Daughaday, W. H.: Sulfation factor: a serum component mediating the action of growth hormone in stimulating incorporation of sulfate into cartilage. J. Clin. Invest. 1956; 35:733.

[4] Chochinov, R. H., and Daughaday, W. H.: Current concepts of somatomedin and other biologically related growth factors. Diabetes 1976; 35:733.

[5] Zapf, J., Schoenle, E., and Froesch, E. R.: Insulin-like growth factors I and II: some biological actions and receptor binding characteristics of two purified constituents of nonsuppressible insulin-like activity of human serum. Eur. J. Biochem. 1978; 87:285–96.

[6] Rinderknecht, E., and Humbel, R. E.: The amino acid sequence of human insulin-like growth factor I and its structural homology with proinsulin. J. Biol. Chem. 1978; 253:2769–76.

[7] Rinderknecht, E., and Humbel, R. E.: Primary structure of human insulin-like growth factor II. FEBS Lett. 1978; 89:283–86.

[8] Zapf, J., Walter, H., and Froesch, E. R.: Radioimmunological determination of insulin-like growth factors I and II in normal subjects and in patients with growth disorders and extrapancreatic tumor hypoglycemia. J. Clin. Invest. 1981; 68:1321.

[9] Hintz, R. L., Liu, R., Marshall, L. B., and Chang, D.: Interaction of somatomedin C with an antibody directed against the C-peptide region of the insulin-like growth factor I. J. Clin. Endocrinol. Metab. 1980; 50:405–406.

[10] Hintz, R. L., and Liu, F.: Demonstration of specific plasma protein binding sites for somatomedin. J. Clin. Endocrinol. Metab. 1977; 45:988–95.

[11] Furlanetto, R. W.: The somatomedin C binding protein: evidence for a heterologous subunit structure. J. Clin. Endocrinol. Metab. 1980; 51:12–19.

[12] Zapf, J., Morell, B., Walter, H., Laron, Z., and Froesch, E. R.: Serum levels of insulin-like growth factor (IGF) and its carrier protein in various metabolic disorders. Acta Endocrinol. 1980; 95:505–17.

[13] Merimee, T. J., Zapf, J., and Froesch, E. R.: Insulin-like growth factors: studies in diabetics with and without retinopathy. N. Engl. J. Med. 1983; 309:527–30.

[14] Gardner, D. F.: In Control: A Patient's Guide to Diabetes Management. Indianapolis, Bio-Dynamics, Inc., 1982.

[15] Boden, G., Master, R. W., Gordon, S. S., Sheman, C. R., and Owen, O. E.: Monitoring metabolic control in diabetic outpatients with glycosylated hemoglobin. Ann. Intern. Med. 1980; 92:357–60.

[16] Mehl, T. D., Wenzel, S. E., Russell, B., Gardner, D. F., and Merimee, T. J.: Comparison of two indices of glycemic control in diabetics: glycosylated serum protein and hemoglobin. Diabetes Care 1983; 6:34–39.

[17] Kennedy, A. L., Mehl, T. D., and Merimee, T. J.: Nonenzymatic glycosylated serum protein: spurious elevation due to free glucose in serum. Diabetes 1979; 29:413–15.

[18] McFarland, K. K., Catalano, E. W., Day, J. F., Thorpe, S. R., and Baynes, J. W.: Nonenzymatic glycosylation of serum proteins in diabetes mellitus. Diabetes 1979; 28:1011–14.

[19] Poulsen, J. E.: Houssay phenomenon in man: recovery from retinopathy in a case of diabetes with Simmond's disease. Diabetes 1953; 2:7–12.

[20] Lundbaek, K., Malmros, R., and Anderson, H. C.: Hypophysectomy for diabetic angiopathy: a controlled clinical trial. *In* Symposium on the Treatment of Diabetic Retinopathy. Goldberg, M. F., and Fine, S. L., Eds. Washington, D.C., Public Health Service publ. no. 1890, 1969:291.

[21] Lundbaek, K.: Growth hormone's role in diabetic microangiopathy. Diabetes 1976; 25 (Suppl. 2):845–49.

[22] Almer, L. O., Pandolfi, M., and Nilsson, I. M.: Diabetic retinopathy and the fibrinolytic system. Diabetes 1975; 24:529–34.

[23] Colwell, J. A., Halushka, P. V., Sarji, K., Levine, J., Sagel, J., and Nair, R. M.: Altered platelet function in diabetes. Diabetes 1976; 25 (Suppl. 2):831–36.

[24] Badawi, H., El-Sawy, M., and Mikhail, M.: Platelet coagulation and fibrinolysis in diabetic and nondiabetic patients with quiescent coronary heart disease. Angiology 1970; 21:511–19.

[25] Merimee, T. J., Fineberg, S. E., and Hollander, W.: Vascular disease in the chronic HGH-deficient state. Diabetes 1973; 22:813–19.

[26] Merimee, T. J., Fineberg, S. E., and McKusick, V. A.: Diabetes mellitus and sexual ateliotic dwarfism: a comparative study. J. Clin. Invest. 1970; 49:1096–1102.

[27] Horner, Y. M., Kemp, S. E., and Hintz, R. L.: Growth hormone and somatomedin in insulin dependent diabetes mellitus. J. Clin. Endocrinol. Metab. 1981; 53:1148–53.

[28] Winter, R. J., Phillips, L. L., Klein, M. N., Traisman, H. S., and Green, O. C.: Somatomedin activity and diabetic control in children with insulin-dependent diabetes. Diabetes 1978; 28:952–54.

[29] Cohen, M. P., Josti, K., and Rye, D. L.: Somatomedin in insulin-dependent diabetes mellitus. J. Clin. Endocrinol. Metab. 1977; 45:236–39.

[30] Tamborlane, W. V., Hintz, R. L., Bergman, M., Genel, M., and Felig, P.: Insulin-infusion pump treatment of diabetes. N. Engl. J. Med. 1981; 305:303–307.

[31] Yde, H.: The growth hormone dependent sulfation factor in serum from patients with various types of diabetes. Acta Med. Scand. 1969; 186:293–97.

[32] Blethen, S. L., Sargeant, D. T., Whitlow, M. G., and Santiago, J. V.: Effect of pubertal stage and recent blood glucose control on somatomedin C in children with insulin-dependent diabetes mellitus. Diabetes 1981; 30:868–71.

[33] Phillips, L. S., Belasky, D. C., and Reichard, L. A.: Nutrition and somatomedin. V. Action and measurement of somatomedin inhibitors in serum from diabetic rats. Endocrinology 1979; 104:1513–18.