Nutrition and Somatomedin

III. Diabetic Control, Somatomedin, and Growth in Rats

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SUMMARY

Observations of a growth hormone-resistant decrease in somatomedin activity associated with conditions of insulinopenia suggest that somatomedin and growth might be related to metabolic control in diabetes mellitus. We examined this relationship by comparing measures of insulin effect with serum somatomedin activity (porcine cartilage bioassay), cartilage growth activity (SO_4 uptake in vitro), and change in body weight in streptozotocin-diabetic rats. A range of metabolic control was produced by administration of varied quantities of insulin over two- and four-day periods. Serum somatomedin activity was significantly correlated with serum glucose and β-hydroxybutyrate ($r = -0.69$ and -0.76, respectively, $p < 0.001$ for both) but not with free fatty acids. A level of serum glucose <240 mg./dl. or β-hydroxybutyrate <1.3 mM at sacrifice was generally associated with serum somatomedin activity within the normal range. Urine glucose levels less than 0.1 gm./24 hr. were also associated with normal serum somatomedin activity. Insulin therapy sufficient to provide β-hydroxybutyrate less than 1.3 mM at sacrifice was associated with levels of serum somatomedin activity and cartilage growth activity comparable to those of normal animals and significantly greater than those of untreated diabetic animals. This level of insulin effect also permitted diabetic animals to gain weight at a rate comparable to that of normal animals.

These studies demonstrate a close relationship between insulin efficacy, serum somatomedin activity, cartilage growth activity, and weight gain and support the hypothesis that through somatomedin, insulin may contribute to growth. DIABETES *26*:864-69, September, 1977.

Growth failure may be a complication of diabetes in children, although the mechanism is not well understood. Since circulating metabolic fuels are abundant, poor growth appears to be due to inadequate utilization of nutrients for growth. Growth-related utilization of metabolic fuels cannot be limited by lack of growth hormone (GH) since plasma levels of GH are normal to elevated.[1] Growth impairment has been associated particularly with inadequate diabetic control with insulin,[2,3] and it seems likely that poor growth in diabetic children is a consequence of insulin deficiency. Since at physiologic levels insulin has little ability to stimulate growth cartilage directly,[4] poor growth due to lack of insulin must be mediated by other factors.

A considerable portion of hormone-directed skeletal growth is attributed to stimulation of growth cartilage by a serum factor (or factors), somatomedin[5] (previously named sulfation factor). Somatomedin in serum is generally measured with bioassays by the ability of serum samples to stimulate sulfate incorporation into cartilage chondroitin sulfate or incorporation of nucleic acid into cartilage DNA or RNA.[6] The existence of somatomedin was originally proposed to explain the growth-promoting actions of GH,[7] and early studies characterized somatomedin primarily as a GH-dependent factor.[8] However, there is now evidence that somatomedin may also be regulated by nutritional status and by insulin.

The importance of nutrition and insulin in the modulation of serum somatomedin activity was suggested initially by clinical observations of low serum somatomedin activity in children with malnutrition and poor growth despite high GH[9] and of normal somatomedin activity in children with obesity and good growth despite low GH.[10] Subsequently, it

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was demonstrated in rats that serum somatomedin activity and cartilage growth activity are closely linked to food intake.[11] Further studies revealed that streptozotocin-induced diabetes in rats is associated with a profound decrease in serum somatomedin activity and cartilage growth activity.[12] Since insulin therapy ameliorated the condition, decreased somatomedin activity was attributed to insulin deficiency.[12]

The studies described above suggested a possible relationship between somatomedin and the efficacy of insulin therapy. In order to quantitate the relation between insulin therapy, diabetic control, somatomedin, and growth, we compared measures of metabolic control with alterations in serum somatomedin activity, cartilage growth activity, and change in body weight in insulin-treated streptozotocin-diabetic rats.

MATERIALS AND METHODS

The procedures for animal care, streptozotocin induction of diabetes, measurements of cartilage growth activity and serum somatomedin activity, and general experimental design have been described in detail previously.[12] They are outlined briefly below.

Young male Charles River rats were given streptozotocin 250-300 mg./kg. I.V. and placed in metabolic cages. Body weight was measured daily and weight change expressed as Δ gm./24 hr. prior to sacrifice. The animals' metabolic status was monitored by semiquantitative estimation of glucose (Clinitest) and ketones (Acetest) in daily 24-hour urine collections and by plasma measurements as described below. At sacrifice, serum somatomedin activity was measured with a bioassay utilizing immature porcine costal cartilage and results expressed as U./ml. compared with a standard serum. (Bioassayable somatomedin activity as measured here is assumed to reflect in vitro the sum of the actions of circulating stimulatory and inhibitory factors that regulate growth cartilage in vivo; the relative sensitivity of this bioassay to stimulation by different somatomedin peptides has not been determined.) Cartilage "growth activity" (as stimulated by somatomedin) was estimated by SO_4 incorporation by the animals' cartilage in vitro and expressed as per cent of control values. Serum glucose was determined with the Beckman glucose analyzer, and β-hydroxybutyrate and free fatty acids (FFA) were measured as previously described.[13,14]

One day after administration of streptozotocin all animals had weight loss, and urine glucose and ketones averaged ≥ 5 gm./dl. and negative, respectively. Insulin was usually begun at this time, although in some experiments groups of rats were sacrificed after a second day of diabetes as untreated controls. Insulin was given in high and low dosage by two schedules to produce varying degrees of diabetic control short of hypoglycemia and ketoacidosis:

Schedule A. Diabetic control maintained or improving: Beginning on day 1 (after streptozotocin), groups of animals received daily subcutaneous injections of protamine zinc insulin (PZI) in either high (10-13 U./100 gm.) or low (2-3 U./100 gm.) dosage. The animals were sacrificed on day 3 after two days of insulin therapy.

Schedule B. Diabetic control maintained or worsening: Beginning on day 1, all animals received daily injections of PZI (9.5-10.5 U./100 gm.) to produce uniform weight gain and eliminate glucose and ketones from the urine. After two days of PZI, on day 3 treatment was begun with twice-daily injections of NPH insulin in either high (2-4 U./100 gm.) or low (0.5-1.5 U./100 gm.) dosage. These animals were sacrificed on day 5 after two days (four injections) of NPH therapy.

Eight experiments, each employing 24 to 30 animals, were performed. Groups of control and experimental animals were sacrificed at different time points during the course of insulin therapy. Within individual experiments at each time point, measurements of weight change, somatomedin activity, and cartilage growth activity were averaged to provide an individual mean for each experimental group; glucose, β-hydroxybutyrate, and FFA were determined in sera pooled from each experimental group. Statistical differences between means were determined by two-tailed grouped *t*-test using separate or pooled variance estimates according to the homogeneity of variance within the test sample.

RESULTS

The general effects of insulin therapy in our diabetic animals are shown in figures 1 and 2. One day after administration of streptozotocin, serum glucose and β-hydroxybutyrate rose significantly above control levels, with a corresponding fall in weight change, cartilage uptake of sulfate, and serum somatomedin activity. Two days of insulin begun at that time (Schedule A, see METHODS) in high dosage to improve diabetic control (figure 1) returned glucose, β-hydroxbutyrate, somatomedin activity, and weight change to levels generally comparable with

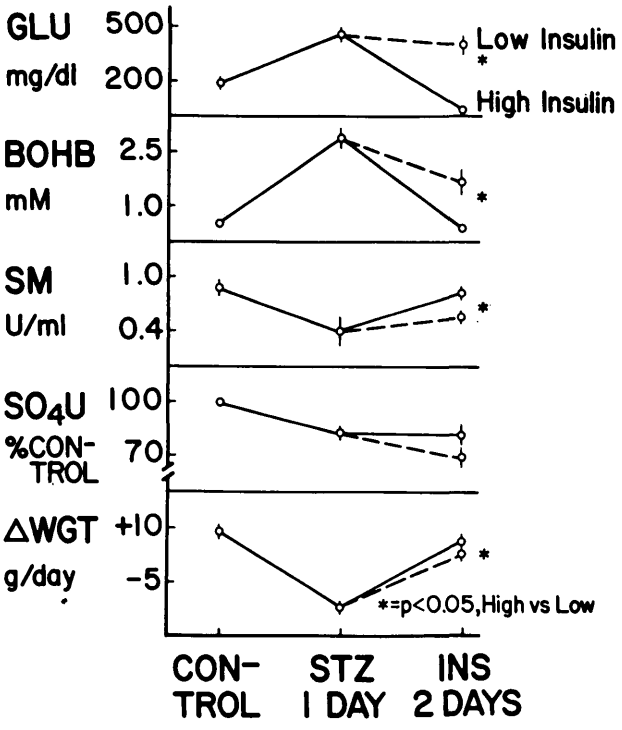

FIG. 1. Serum glucose, serum β-hydroxybutyrate, serum somatomedin activity, cartilage growth activity, and change in weight in streptozotocin-diabetic rats treated with insulin for two days as in Schedule A (see METHODS). Mean ± S. E. M. for four experiments.

controls' and maintained cartilage uptake of sulfate. Low-dose insulin to maintain control produced intermediate levels of the metabolic and growth-related parameters that were generally significantly different from those produced by high-dose insulin. In other experiments (Schedule B), diabetic animals were treated uniformly with insulin for two days to produce good control (figure 2), generally comparable with that of the high-insulin animals treated as in schedule A. Two subsequent days of insulin in high dosage to maintain control kept glucose, β-hydroxybutyrate, somatomedin activity, and weight change at control levels and increased cartilage sulfate uptake. Insulin in lower dosage to decrease control increased glucose and β-hydroxybutyrate, decreased somatomedin activity and weight change, and maintained cartilage sulfate uptake. Since the only biologically significant differences between the two schedules of insulin therapy were found in cartilage sulfate uptake—still below control levels after two days of insulin but normalized after four days of insulin—the data were pooled for analysis of the effects of diabetic control on somatomedin and growth.

Within the population of control, diabetic, and insulin-treated diabetic animals, correlations among measures related to growth (serum somatomedin activity, cartilage growth activity, weight change) and measures related to metabolic control (serum glucose, serum β-hydroxybutyrate, and FFA) are shown in table 1. Serum somatomedin activity, cartilage growth activity, and weight change were significantly related, as were serum glucose, β-hydroxybutyrate, and FFA. Serum somatomedin activity and cartilage growth activity were correlated more with serum glucose and β-hydroxybutyrate than with FFA, although

TABLE 1

Correlations among measures of insulinization and parameters related to growth: serum glucose, serum β-hydroxybutyrate, serum free fatty acids, serum somatomedin activity, cartilage growth activity (SO_4U: SO_4 uptake in vitro), and change in weight (ΔWGT: gain or loss in weight over the 24 hours prior to sacrifice) in rats from eight separate experiments

	SO_4U	ΔWGT	Glucose	β-OHB	FFA
Somatomedin	0.65‡	0.66‡	−0.69‡	−0.76‡	−0.35*
SO_4U	—	0.44†	−0.40†	−0.78‡	−0.24*
ΔWGT		—	−0.74‡	−0.70‡	−0.82‡
Glucose			—	0.69‡	0.76‡
β-OHB				—	0.82‡

*Denotes $p > 0.05$, †denotes $p < 0.01$, and ‡denotes $p < 0.001$.

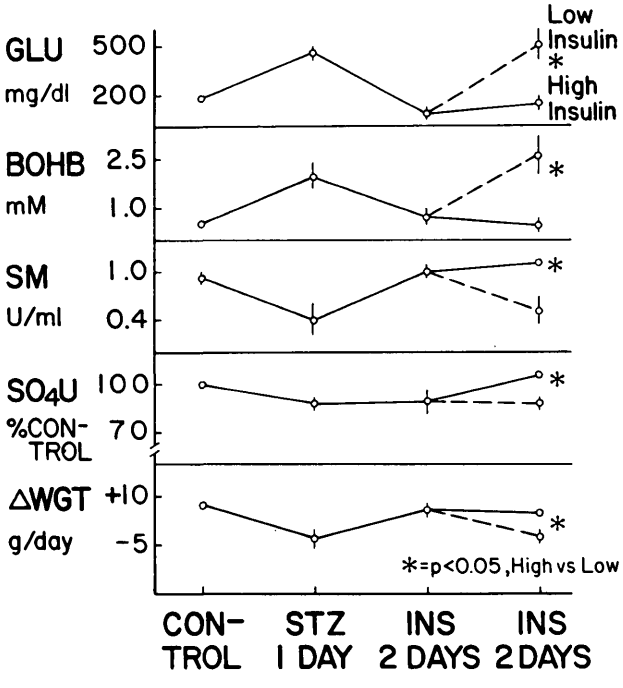

FIG. 2. Serum glucose, serum β-hydroxybutyrate, serum somatomedin activity, cartilage growth activity, and change in weight in streptozotocin-diabetic rats treated with insulin for four days as in Schedule B (see METHODS). Mean ± S.E.M. for four experiments.

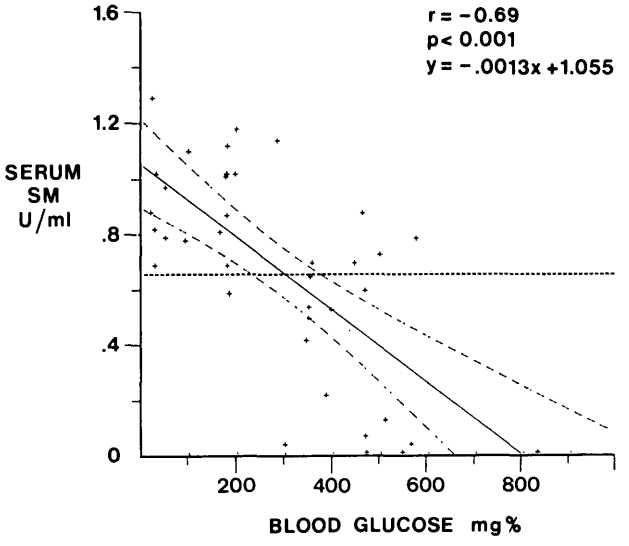

FIG. 3. Serum somatomedin activity vs. serum glucose at sacrifice in control and diabetic rats from eight experiments. Shown is the least-square regression line with 95 per cent confidence envelopes. The horizontal line indicates the lower limit of normal (0.65 U./ml.).

weight change was correlated significantly with all three measures of metabolic control. The more important associations are illustrated in figures 3, 4, and 5.

Serum somatomedin activity correlated significantly with serum glucose (at sacrifice), as shown in figure 3. The lower 95 per cent confidence limit of the linear regression intersected a somatomedin activity of 0.65 U./ml. at a glucose value of 240 mg./dl. Thus, within this sample population a level of serum glucose less than 240 mg./dl. was generally associated with a level of serum somatomedin activity within the normal range (0.65-1.35 U./ml.). Since significant glycosuria (> 0.1 gm./24 hr.) occurred when serum glucose was greater than 300 mg./dl. (not shown), the level of urine glucose provided a convenient assessment of the relation of serum somatomedin activity to diabetic control: glycosuria was associated with decreased serum somatomedin activity (figure 4).

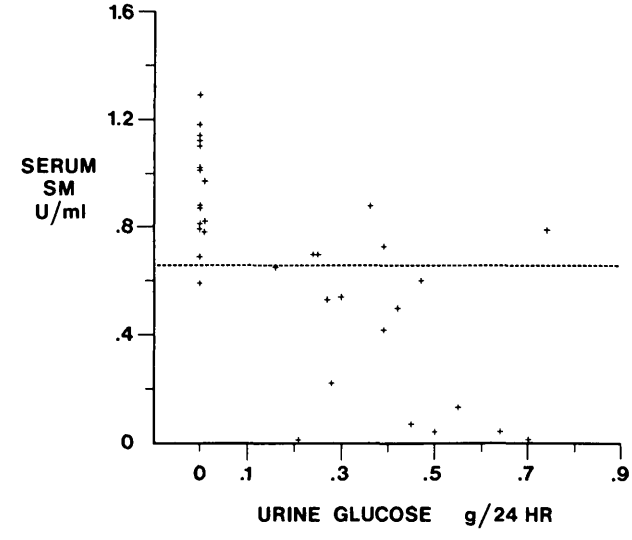

FIG. 4. Serum somatomedin activity vs. urine glucose over the 24 hours prior to sacrifice in control and diabetic rats from eight experiments.

Serum somatomedin activity also correlated significantly with serum β-hydroxybutyrate, both over the entire group of experimental animals (figure 5) and within the smaller population, consisting only of insulin-treated diabetic rats ($r = -0.74$, $p < 0.001$, regression $y = -0.21x + 1.04$; see figure 5). In both groups, a level of β-hydroxybutyrate less than 1.3 mM was associated with a level of serum somatomedin activity within the normal range. The effect of such a degree of control on other metabolic and growth-related measures is shown in figure 6. In insulin-treated diabetic animals with serum β-hydroxybutyrate less than 1.3 mM, levels of serum glucose, serum somatomedin activity, and cartilage growth activity were comparable to those of control animals and significantly different from levels in untreated diabetic animals. Moreover, this level of diabetic control in animals treated with insulin over a four-day period permitted weight gain parallel to that

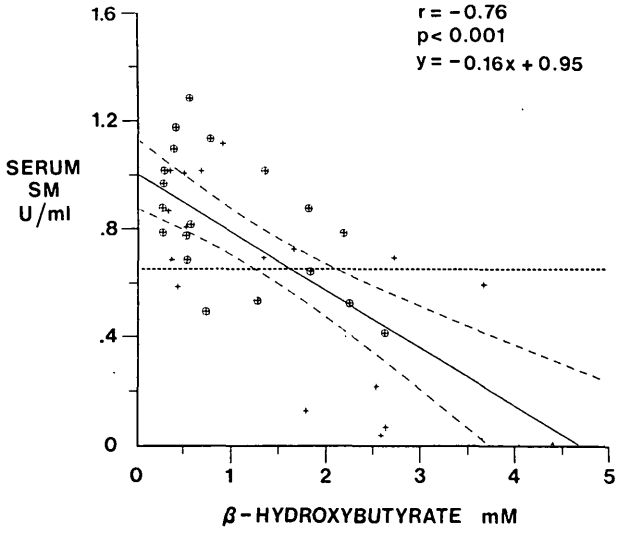

FIG. 5. Serum somatomedin activity vs. serum β-hydroxybutyrate in control and diabetic rats from eight experiments. Insulin-treated diabetic rats are designated by ⊕. Data shown as in figure 3.

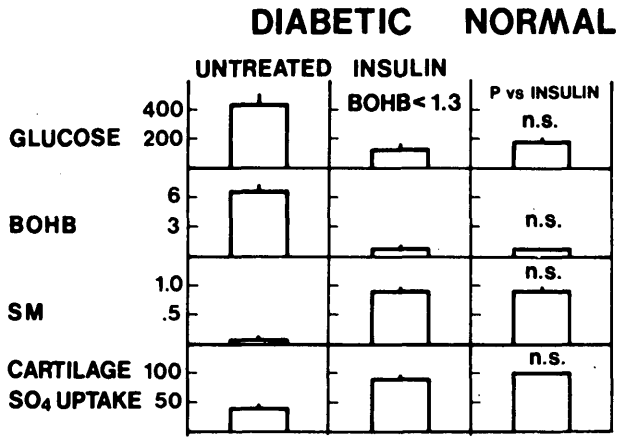

FIG. 6. Effect of insulin therapy of diabetes sufficient to provide serum β-hydroxybutyrate less than 1.3 mM at sacrifice. Shown are levels of serum glucose, serum somatomedin activity, and cartilage growth activity for insulin-treated diabetic rats with this degree of metabolic control, untreated diabetic rats, and control rats. Also shown is the probability of difference between insulin-treated rats and control rats. Mean ± S.E.M.

of control animals ($p > 0.1$ for nonparallelism) (figure 7). Actual weight gain in these animals included a 15 per cent increase above weight prior to the onset of diabetes and thus could not be attributed simply to the regain of body fluid and electrolyte that was lost before insulin therapy was begun. This suggests that insulin therapy sufficient to normalize serum somatomedin activity was also sufficient to permit normal growth.

DISCUSSION

Poor growth was a common feature of juvenile diabetes prior to the availability of insulin.[15] Although insulin therapy has permitted normal growth in many diabetic children, such therapy is not always successful. In particular, growth impairment is more frequent when diabetic control is poor.[2,3] Regardless of the specific etiology of poor control, its common manifestations—hyperglycemia, glycosuria, ketonuria, and frequent episodes of ketosis—are due to insulin insufficiency.

Although insulin in pharmacologic concentrations provides significant direct stimulation of growth cartilage,[4] it seems more likely that insulin contributes to growth indirectly by promoting the generation of somatomedin. Our studies in streptozotocin-diabetic rats support such a hypothesis,[12] and additional evidence is provided by studies of perfused livers in which direct addition of insulin enhanced hepatic release of somatomedin activity.[16] (As noted above [see METHODS], bioassayable somatomedin activity is a measure of growth cartilage stimulation, which is due predominantly to somatomedin peptides but may reflect other factors as well; the relative contribution of the different somatomedins is unknown.) If indeed insulin influences growth via somatomedin, there should be a relation between the degree of insulin effectiveness (as shown by metabolic control of diabetes) and somatomedin activity in the circulation.

Early measurements by Jensen et al.[17] suggested that serum somatomedin activity in human diabetics was normal prior to treatment and unaffected by dietary therapy. However, in subsequent studies of a larger group of patients, Yde[18] reported decreased somatomedin activity both in newly diagnosed diabetics and in insulin-treated patients with diabetic retinopathy. In addition, Yde found that somatomedin activity was inversely related to serum glucose in nonobese diabetics but not in obese diabetics. The discrepancy between these studies may be due in part to methodologic problems in somatomedin measurement, to Jensen's study of patients with relatively good control and a narrow range in glucose values, and to differences in sample size. More recently, Van den Brande[10] reported that serum somatomedin activity was in the low-normal range in five untreated juvenile diabetics but did not reveal the magnitude of their metabolic abnormalities. Although these studies suggested that somatomedin might be influenced by diabetic control, there was no attempt to relate serum somatomedin activity to measures of control other than serum glucose, and there was no attempt to define a degree of control with insulin that would be

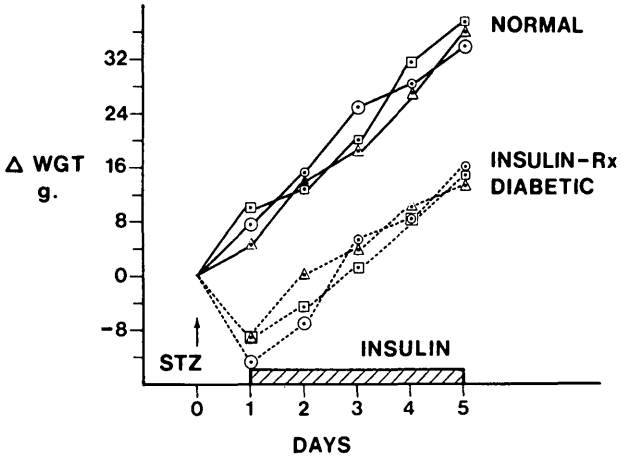

FIG. 7. Weight change of control and insulin-treated diabetic rats in three experiments in which four days of insulin therapy produced serum β-hydroxybutyrate less than 1.3 mM at sacrifice of the diabetic animals.

associated with normal levels of somatomedin activity and normal growth.

In this study of diabetes in rats, serum somatomedin activity was inversely related to serum glucose and β-hydroxybutyrate as measures of insulin-induced diabetic control. At sacrifice, levels of serum glucose less than 240 mg./dl. and/or serum β-hydroxybutyrate less than 1.3 mM were generally associated with serum somatomedin activity within the normal range. Such a degree of control with insulin permitted normalization of cartilage growth activity and, over a four-day period, weight gain comparable to that of control animals. Semiquantitative estimation of urine glucose provided a simple measure of insulin efficacy that was closely related to serum somatomedin activity; glycosuria greater than 0.1 gm./24 hr. (usually with serum glucose greater than 300 mg./dl.) was generally associated with reduced serum somatomedin activity. The correlation of serum somatomedin activity with FFA fell short of significance ($p < 0.06$), perhaps in part because of regulation of FFA by insulin that did not parallel entirely the regulation of somatomedin.

In combination, these studies demonstrate a close relation between biologic effectiveness of exogenous insulin (metabolic control of diabetes), serum somatomedin activity, cartilage growth activity, and weight gain. These findings support the hypotheses (a) that insulin is an important regulator of somatomedin, and (b) that through somatomedin, insulin contributes to growth. Our data also suggest that it may be possible to determine a degree of control for human diabetes that would provide a reasonable likelihood of normal somatomedin generation and normal growth. Measurements of somatomedin activity in the serum of diabetic children might then provide a useful marker to indicate that such control had been achieved.

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