Effect of Continuous Low-Dose Insulin Treatment on Subsequent Incidence of Diabetes in Genetically Prediabetic Chinese Hamsters

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SUMMARY

In an attempt to prevent the onset of diabetes, young, genetically prediabetic (but not yet hyperglycemic) Chinese hamsters were treated continuously with insulin via minipump for 4 wk beginning 1 wk before the predicted age of onset of glucosuria (age 7 wk). Continuous insulin infusion, which increased the plasma insulin levels by 70%, did not cause hypoglycemia, nor did it reduce the incidence or severity of diabetic symptoms over the ensuing year of observation. In fact, early treatment with exogenous insulin tended to cause increased hyperglycemia and glucosuria. No plasma anti-insulin antibodies were detected 3 and 9 months after stopping insulin treatment. DIABETES 28:544–547, June 1979.

Soon after the onset and treatment of juvenile-type diabetes in humans, a period of decreased insulin requirement is sometimes seen. This remission or honeymoon is characterized by increased endogenous insulin.[1,2] Careful control of blood glucose by continuous insulin infusion (the artificial β-cell, etc.) for brief periods can also lead to improved endogenous insulin release;[3] however, such remissions are usually not permanent and long-term studies are not available.

The present study was designed to determine if continuous treatment with insulin of genetically prediabetic Chinese hamsters (i.e. before the onset of symptoms) could modify their subsequent incidence of diabetes. Certain sublines of these highly inbred animals exhibit an insulin-deficient nonobese type of diabetes with many sequelae similar to those seen in juvenile-onset diabetes in humans.[4] 95% of the hamsters from the X diabetic subline show glucosuria with a mean age of onset of 6.9 wk (SD = 2.9; N = 80) (personal communication, G. C. Gerritsen, The Upjohn Company, Kalamazoo, Michigan).

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MATERIALS AND METHODS

26 animals of both sexes from the diabetic X subline (inbred 18 to 20 generations) were shipped to our laboratories over a 13-wk period at age 5–6 wk from the colony at The Upjohn Company, Kalamazoo, Michigan. Except as otherwise noted, blood for glucose and hormone measurements was drawn between 1000 and 1100 h from the orbital sinus of fed animals. Only prediabetics with mean nonfasting plasma glucose levels still below 120 mg/dl that had not yet shown glucosuria were included in the study (6 of 26, or 23%, were thus disqualified). Although not significant statistically, the mean blood glucose levels of both experimental and control prediabetic groups at weeks 5 and 6 were greater than the normals' mean level. Thus, the prediabetics may have already progressed into the early stages of diabetes by the time infusion of insulin (N = 10) or saline solution (N = 10) was begun at week 6. Six age-matched normal M subline hamsters (inbred 20 or more generations) were treated with saline solution as an additional control. Methods for determination of insulin antibody titers[5] and plasma and urine glucose levels[6] have been previously described, as have the use of the Alzet minipump, Alza Corporation, Palo Alto, California, for infusion of insulin or saline solution[5,7] and the procedure for retroorbital venous puncture.[8] Each minipump (an implantable, 6.5 mm × 2.5 cm, self-contained, 1-wk infusion device) was filled with single-component pork insulin, Eli Lilly, Lot 615-1082B-17I, pH 6-7, in saline solution containing 1% albumin (or saline/albumin alone as control) so as to release 5 U/kg body wt/day. This dosage was chosen because, in a previous study,[5] 10 U/kg body wt/day given via minipump caused hypoglycemia in mildly diabetic, older hamsters. Four pumps were sequentially implanted subcutaneously in each animal. The concentration of insulin was changed each week to maintain the same dosage with respect to the animals' increasing weight. A single-time-point (minute 20) fast/refeed (or meal) tolerance

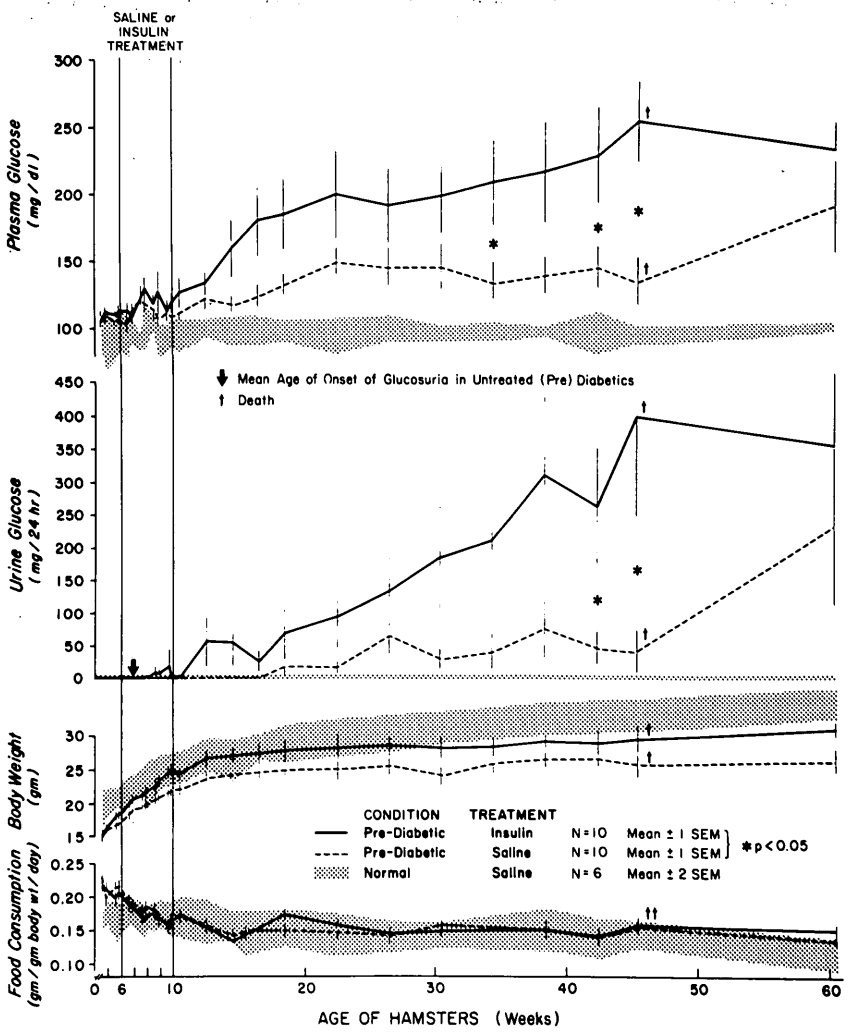

FIGURE 1. Effect of 4-wk insulin (5 U/kg body wt/day) or saline treatment on nonfasting plasma and urine glucose, body weight, and food consumption of genetically prediabetic Chinese hamsters. Age- and sex-matched, genetically normal hamsters received saline solution alone as an additional control. All animals were followed for 1 yr after stopping treatment. (Probability values calculated by two-tailed Student's *t* test.)

test procedure was adapted from Gerritsen and Blanks[9] as follows: Animals were fasted 24 h with water ad libitum 1130–1130 h; no zero time blood sample was taken; they were fed 200 ± 2 mg of their normal diet (not glucose) and then bled exactly 20 ± 1 min later. All animals consumed the food within 4 min of its presentation. This simple, single sample tolerance test avoids the stress of multiple bleedings, which we have found influence the test results.

Plasma insulin and glucagon were measured with a micromodification of a single-antibody, charcoal-precipitation radioimmunoassay. For both assays, small, round-bottomed wells in 250-μl Microtiter "U" polyvinyl well-plates were used (Cooke Laboratory Products, Division of Dynatech Laboratories, Alexandria, Virginia).

For the glucagon assay, the wells were first coated with albumin as follows: They were filled with 1% bovine serum albumin (BSA) in 0.9% saline; left to stand 5 min; aspirated at room temperature; and then air-dryed overnight at 4 °C. The following were then added: 20 μl plasma (containing 3 mg/ml EDTA and 500 KIU/ml Trasylol) or glucagon standards (0–800 pg/ml); 40 μl glucagon antiserum, appropriately diluted; and 50 μl ^{125}I-labeled glucagon (approximately 9 pg/50 μl, or 0.4 nCi)—all prepared in 0.2 M glycine buffer (pH 8.8) containing 0.25% human serum albumin and 1% lamb serum. For no-antibody controls, 40 μl of this buffer replaced the 40 μl of glucagon antiserum. After 96 h at 4 °C, 50 μl of a charcoal-dextran solution (0.5% charcoal and 0.25% dextran in 0.2 M glycine buffer) and 20 μl of lamb serum were added. The standards received 20 μl glycine buffer in place of the lamb serum. The plates were centrifuged for 30 min at 4 °C and the supernatants were aspirated. The wells were then cut apart and each was counted in a gamma counter. Plasma samples, standards, and no-antibody controls were assayed in duplicate or triplicate.

The following modifications in this procedure were made for the assay of plasma insulin levels. Only 5-μl aliquots of plasma or 10-μl insulin standards (0–400 μU/ml) were used. Pork insulin standards were prepared in 0.2 M glycine buffer (pH 8) containing 0.5% BSA and 0.5% gamma globulin. Only 20-μl aliquots of antiserum, appropriately diluted, were used. For trace, 50 μl (10 pg/50 μl, or approximately 1.0 nCi) ^{125}I-labeled pork insulin was used. Antiserum and trace were diluted in 0.4 M glycine buffer (pH 8) with 1% BSA. For no-antibody controls, 20 μl of the 0.4 M glycine buffer with 1% BSA was used. After only 24–48 h of incubation, charcoal precipitation was done with 100 μl of a 1% charcoal solution in a buffer composed of 18 parts of 0.9% saline, one part 0.1 N HCl, and one part buffer base (142.7 mM sodium barbital, 142.7 mM sodium acetate; pH 7.4). Lamb serum was not used with the charcoal in the insulin assay.

TABLE 1
Plasma insulin and glucagon levels, insulin antibody titers, and meal tolerance tests in Chinese hamsters*

Group	Age			
	10 wk†	23–26 wk	43–51 wk	63–76 wk
Plasma insulin (μU/ml)				
Prediabetic insulin-treated	109 ± 6 (7) [P < 0.01]	105 ± 18 (6) [NS]	118 ± 16 (10) [NS]	
Prediabetic saline-treated	64 ± 7 (7) [NS] P < 0.001	111 ± 18 (6) [NS] NS	119 ± 14 (10) [NS] NS	
Normal (saline)	74 ± 8 (4)	145 ± 25 (3)	105 ± 8 (3)	
Plasma glucagon (pg/ml)				
Prediabetic insulin-treated		188 ± 25 (6) [NS]	197 ± 7 (10) [P < 0.02]	
Prediabetic saline-treated		188 ± 35 (6) [NS] NS	196 ± 6 (10) [P < 0.05] NS	
Normal (saline)		209 ± 48 (3)	164 ± 10 (6)	
Anti-insulin antibody titers				
Prediabetic insulin-treated		0 ± 0 (10)	0 ± 0 (10)	
Prediabetic saline-treated		0 ± 0 (6)	0 ± 0 (10)	
Normal (saline)		0 ± 0 (3)	0 ± 0 (6)	
Meal tolerance test‡ (plasma glucose in mg/dl)				
Prediabetic insulin-treated				207 ± 11 (9) [P < 0.005]
Prediabetic saline-treated				180 ± 10 (9) [NS] NS
Normal (saline)				152 ± 9 (6)

* Data given as mean ± SEM, with the number of animals given in parentheses. Significance of difference when compared with normals calculated by two-tailed Student's *t* test and given in square brackets; NS = not significant. Difference between insulin- and saline-treated prediabetics is shown. Trasylol and EDTA added to plasma to give a final concentration of 500 KIU/ml and 3 mg/ml, respectively, for hormone and antibody plasma samples. Blood for hormone and antibody measurements was drawn from fed animals between 1000 and 1100. Meal tolerance test followed a 24-h fast.

† During 4th wk of insulin or saline treatment.

‡ See METHODS.

RESULTS

As seen in Figure 1, the normal hamsters' mean plasma glucose levels rarely rose above 100 mg/dl throughout the 60-wk study. After the initial growth spurt, which ended at 10–20 wk, all animals showed stable body weights and food consumption. In agreement with the urine TesTape studies at Upjohn, the saline-treated prediabetics first showed markedly increased glucosuria at around 7 wk of age. Consistent with findings at Upjohn of reduced life span in the genetically diabetic animals,[10] two of the diabetics died at age 45–60 wk while none of the normals had died in that period.

Insulin treatment significantly increased the immunoassayable plasma insulin levels in the prediabetics (Table 1) but did not cause hypoglycemia, nor did it postpone or prevent the onset of diabetic symptoms (Figure 1) for the subsequent 50-wk observation period. In fact, with increasing age, there was a tendency for the prediabetics that had received insulin to show greater hyperglycemia and greater glucosuria than the saline-treated prediabetics (see asterisks, Figure 1). A single-time-point, meal tolerance test 12–15 months after cessation of treatment (Table 1) also showed a higher plasma glucose level among the insulin-treated diabetics as compared with the saline-treated animals ($0.05 < P < 0.1$). Three and nine months after cessation of treatment (Table 1), the diabetic hamsters showed plasma insulin and glucagon levels that were not consistently different from the normal animals' and not affected by the previous treatment. Also, there were no detectable anti-insulin antibodies in the insulin-treated animals' plasma.

DISCUSSION

The present study was designed to see if continuous infusion of insulin during a critical early period in the development of hyperglycemia could reduce the incidence of diabetes.

The data in Figure 1 represent the mean data from 10 animals in each prediabetic group. In each group, one or two animals never showed repeated hyperglycemia or glucosuria and another two or three only intermittently showed grossly elevated plasma and urine glucose levels. Thus, although the animals studied had come from a subline that had been brother/sister inbred for 18 to 20 generations, and therefore was presumably genetically homogeneous,[11] the symptoms of the individual animals varied widely. Since 23% of the prediabetics had been excluded from the study because of hyperglycemia, it was not unwarranted for only 60–70% of the retained animals to become glucosuric instead of the anticipated 95%, or for those retained to be relatively mild diabetics.

4 wk of treatment with continuous insulin during the period of increasing hyperglycemia did not postpone the onset or reduce the severity of the subsequent diabetes.

In fact, there was a tendency for the insulin-treated prediabetics to show greater mean plasma and urine glucose levels than the saline-treated prediabetics. Possibly, a greater suppression of plasma glucose levels at age 7–10 wk or even earlier (so that plasma glucose levels were completely within the normal range) might have reduced the later diabetic symptoms. Also, insulin release from the minipump is constant, and minute to minute regulation of blood glucose is not possible.

It seems unlikely that any exacerbation of the diabetes was caused by an autoimmune attack consequent to the exogenous insulin because no anti-insulin antibodies were found in the animals' plasma samples 3 and 9 months after immunization with the foreign antigen (pork insulin). However, insulin administration has been shown to induce lymphocytic infiltration of the islets,[12] and lymphocytic attack and insulin antibody production do not necessarily occur in parallel. Therefore, autoimmunity is not ruled out.

The early insulin treatment in the present experiment may have reduced the animals' insulin receptors by down regulation.[13] However, such an effect would not be likely to continue months after cessation of insulin treatment.

Gerritsen and Dulin have shown that food restriction of genetically prediabetic hamsters from birth onward markedly reduces the subsequent incidence and severity of diabetic symptoms.[14] The early insulin treatment in the present study may have caused an increased deposition of fat (at age 6–10 wk) and, because overly fat cells tend to be insulin-resistant,[15] may thus have caused insulin resistance and exacerbated diabetic symptoms. The insulin-treated prediabetics tended to be slightly but not significantly heavier than the saline-treated prediabetics. Estimation of percentage of body fat or food restriction or both would be required to rule out the insulin-induced obesity theory. Alternatively, suppression of plasma glucose by an agent that does not stimulate fat deposition, e.g., a biguanide, might have prevented the postulated insulin resistance.

In conclusion, insulin infusion for a period of 4 wk in genetically prediabetic, but not yet hyperglycemic, hamsters at a dosage that did not reduce plasma glucose levels was not able to prevent, postpone, or ameliorate their diabetes.

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