Lack of Glucagon Response to Hypoglycemia in Type I Diabetics After Long-Term Optimal Therapy with a Continuous Subcutaneous Insulin Infusion Pump

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SUMMARY

Counterregulatory hormonal responses were studied in six patients after 4–18 mo treatment with a continuous subcutaneous insulin infusion pump. In response to insulin-induced hypoglycemia, significant increases in epinephrine, norepinephrine, cortisol, and growth hormone were measured in all subjects, while in five of the six patients glucagon levels did not increase at all. The persistence of these abnormal glucagon responses despite long-term optimal glucose control suggests that they are not due to hyperglycemia per se, but are due rather to a specific alpha cell abnormality. The high incidence of asymptomatic hypoglycemia in these patients emphasizes that caution is necessary to avoid serious hypoglycemia when striving for near-normal glucose control with insulin infusion pump therapy. DIABETES 32:398–402, May 1983.

It has recently been demonstrated that near-normal glycemic control can be achieved in type I diabetics using continuous subcutaneous insulin infusion with a portable pump. The optimal glucose control achieved with this form of therapy has been accompanied by improvements in a variety of other metabolic abnormalities present in type I diabetes. This includes reduction in the elevated serum levels of glucagon,[1] growth hormone,[2] lipids, and branch chain amino acids;[3] improvement in growth hormone and catecholamine responses to exercise;[2] and normalization of abnormalities in mineral metabolism.[4] A further hormonal abnormality with important clinical implications in type I diabetics is the diminished or absent glucagon response to hypoglycemia.[5] Because of the critical role that an increase in plasma glucagon is known to play in preventing severe hypoglycemia,[6] it is of importance to ascertain whether this hormone's response will improve coincidentally with the induction of euglycemia. There is evidence that short-term tight glucose control of up to 20 days does not reverse this abnormal glucagon response.[7,8] However, the effect of long-term optimal glucose control on these abnormal responses has not been previously evaluated. We therefore measured changes in plasma glucagon during insulin-induced hypoglycemia in a group of type I diabetics who had been under optimal control during long-term (4–18 mo) therapy with an insulin infusion pump.

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PATIENTS AND METHODS

Patient selection and therapy. Six insulin-requiring diabetics (C-peptide < 0.1 pm/ml after 1 mg glucagon administered intravenously) were selected for therapy with the insulin pump because of suboptimal blood glucose control on conventional insulin therapy. During an initial hospitalization, patients were instructed in home glucose monitoring, meal planning, and the use of the infusion pump (Auto Syringe AS6C, Auto-Syringe Inc., Hooksett, New Hampshire). Insulin (monocomponent pork regular, Eli Lilly, Indianapolis, Indiana) was administered via infusion tubing attached to a 27-gauge needle placed in the subcutaneous tissue over the anterior abdominal wall and secured with tape. Approximately half of the total insulin dose was given as a basal infusion and the other half as premeal boluses. Patients changed the tubing and rotated the injection sites every 48–72 h. They were instructed to perform home blood glucose monitoring four times per day using glucose-oxidase strips (Dextrostix, Ames, Elkhart, Indiana) and a reflectance meter (Dextrometer, Ames). One day every 2 wk patients obtained a profile of seven blood glucose measurements.[9] The glucose results were recorded in a log kept by each patient. The frequency and severity of symptomatic hypoglycemic reactions were also recorded. Outpatient follow-up included at least weekly telephone conversations with members of the medical team and monthly office visits for examination and glycosylated hemoglobin determinations.

Evaluation of autonomic nerve function. The status of the autonomic nervous system was evaluated after overnight recumbency. The following parameters were measured as

previously described:[10] (1) heart rate variation from expiration to inspiration during deep breathing at a rate of 6 breaths/min. A heart rate variation of <10 beats/min was considered abnormal; (2) the ratio of the RR interval measured during expiration and inspiration of 10 deep breaths (E/I ratio). A ratio of <1.1 was considered abnormal; and (3) the ratio of the RR interval of the thirtieth beat to the fifteenth beat after standing (30/15 ratio). A value of <1.03 was considered abnormal.

Experimental protocol. On the day before the insulin hypoglycemia study, patients were admitted to the clinical research center. All subjects gave written informed consent. The protocol was approved by the Clinical Investigation Committee at the University of Chicago.

After an overnight fast the subcutaneous insulin infusion was discontinued. An infusion catheter was inserted into an antecubital vein and a sampling catheter was placed in a retrograde direction into a dorsal vein of the opposite hand with its tip placed as distally as possible. The hand was kept in a warm heating blanket and the oxygen saturation of blood samples obtained from this catheter was >90%, indicating adequate arterialization of the venous sample.

A bolus injection (36 μCi) of 3^3H glucose (New England Nuclear, Boston, Massachusetts) followed by a constant infusion (0.36 μCi/min) was then administered. The infusion was continued for 120 min to ensure complete equilibration of the 3^3H glucose. After a 30-min baseline period, a bolus of insulin (0.025 U/kg) followed by a constant infusion (1.7 mU/kg/min) was administered until the plasma glucose fell to 45 mg/dl. The insulin infusion was then discontinued and the subjects were monitored for spontaneous recovery of plasma glucose for an additional 90 min. Samples for glucose and 3^3H glucose were drawn at 5–10-min intervals throughout the study. Catecholamines, glucagon, epinephrine, growth hormone, cortisol, and free insulin levels were measured at 5–30-min intervals.

Analytic techniques. Plasma glucose was measured in a glucose analyzer (Model 23A, Yellow Springs Instrument Co., Yellow Springs, Ohio). Plasma glucagon[11] and serum free insulin[12] were measured by radioimmunoassay as previously described. Plasma catecholamines were measured using the Upjohn catecholamine radioenzymatic assay kit (Upjohn Chemical Co., Kalamazoo, Michigan).[13] Growth hormone was measured by radioimmunoassay[14] and cortisol by competitive binding assay.[15] Total glycosylated hemoglobin was measured using the thiobarbituric acid method.[16]

3^3H glucose turnover was measured by methods initially described by Wall et al.[17] and simplified by DeBodo.[18] Plasma was deproteinized by the method of Somogyi.[19] The rates of glucose appearance (Ra) and disappearance (Rd) were calculated as reported by Radziuk et al.[20] using a single compartment model with a pool fraction constant of 0.65. The data were smoothed as suggested by Cherrington.[21]

Data analysis. Results are expressed as mean ± SEM. The significance of differences was evaluated using paired or nonpaired two-tailed *t* tests where applicable. P values <0.05 were considered significant.

RESULTS

The relevant clinical information is summarized in Table 1. At the time of study all patients had glycosylated hemoglobin values within the normal range for our laboratory (<8.3%), and this level of control had been achieved for at least 3 mo in those patients who had been on the pump for 4 mo (patient nos. 2, 3, 5) and for at least 10 mo in those patients who had been on the pump for 12–18 mo (patient nos. 1, 4, 6). The mean plasma glucose level obtained by home blood glucose monitoring was 115 mg/dl or less in all patients for at least 3 mo before the time of testing. An evaluation of the frequency of hypoglycemia revealed that 7% of all recorded home blood glucose values were <45 mg/dl, but only approximately one-half of these were associated with symptoms suggestive of hypoglycemia, including sweating, palpitations, anxiety, and excessive hunger. Patient nos. 2, 3, 4, 5, and 6 did not report any symptomatic episodes of neuroglycopenia requiring medical assistance. Patient no. 1 reported a single episode of severe confusion necessitating intravenous glucose administration.

The changes in plasma glucose, glucagon, and epinephrine during and after the acute insulin infusion test are shown in Figure 1. An insulin infusion of 44 ± 7.2 min in duration was necessary to lower the baseline plasma glucose from 109.3 ± 11.7 mg/dl to less than 45 mg/dl. After discontinuing the insulin infusion, the plasma glucose continued to fall in all patients, reaching a nadir of 27 ± 2.4 mg/dl. Patient nos. 1, 2, 3, 4, and 6 showed no evidence of spontaneous recovery of plasma glucose levels. When signs of neuroglycopenia developed, intravenous glucose was administered

TABLE 1
Clinical characteristics of the six patients treated with the insulin pump

Type I diabetics	Sex	Age (yr)	Duration of diabetes (yr)	Autonomic neuropathy	Duration of pump therapy (mo)	Glycosylated hemoglobin* (%)	Mean plasma glucose† (mmol/L)
1	M	41	13	—	12	7.7	106
2	F	27	20	—	4	7.3	113
3	F	29	17	—	4	7.2	90
4	F	26	16	—	18	7.5	115
5	M	28	14	—	4	7.7	112
6	F	25	16	—	12	7.6	110
Mean		29.3	16		9	7.5	107.7
±SEM		±2.4	±1		±2.4	±0.1	±3.7

*Normal glycosylated hemoglobin was <8.3%.
†A profile of seven home glucose values was obtained by each patient one day every 2 wk. The mean plasma glucose was derived from the means of these profiles.

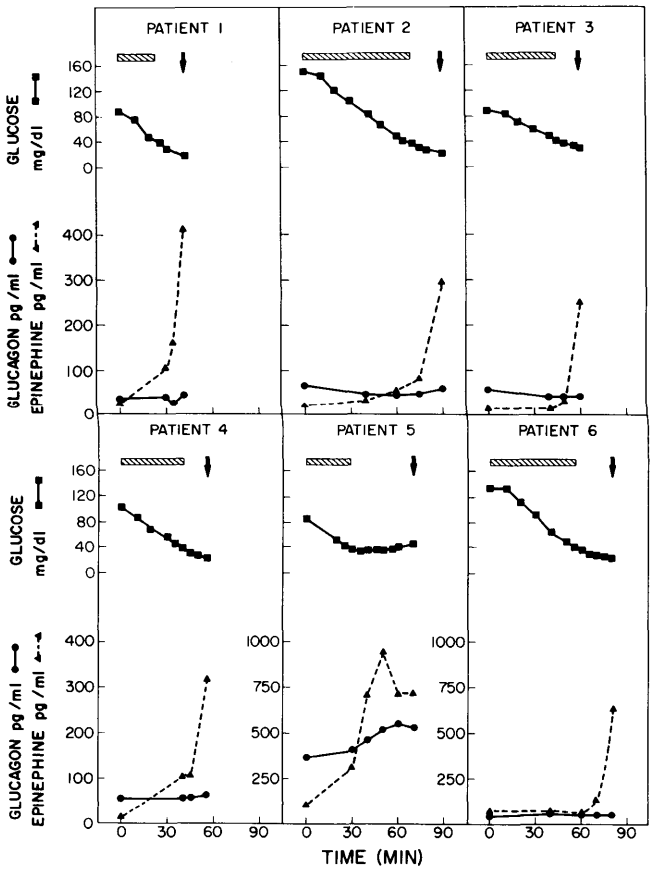

FIGURE 1. The effect of insulin hypoglycemia on plasma glucose and epinephrine in six patients treated with the insulin pump. The duration of insulin infusion is represented by the hatched bars ▨. The arrows indicate the time at which the test was terminated by intravenous glucose (patient nos. 1, 2, 3) or glucagon (patient nos. 4, 5, 6).

to patient nos. 1, 2, and 3. Intravenous glucagon (10 μg bolus + 5 ng/kg/min infusion) instead of glucose was given to patient nos. 4, 5, and 6, resulting in a prompt increase in the plasma glucose levels and reversal of the signs of neuroglycopenia. In patient no. 5, the plasma glucose stabilized at 38 mg/dl, but after a further 40 min it had risen to only 45 mg/dl. Because the patient showed evidence of mild confusion, glucagon was administered intravenously. Within 10 min plasma glucose had risen to 60 mg/dl and neurologic signs had disappeared.

The basal rate of hepatic glucose production (2.3 ± 0.1 mg/kg/min) fell to a nadir of 0.5 ± 0.2 mg/kg/min during the insulin infusion. After discontinuing insulin, the rate rose to 2.5 ± 0.5 mg/kg/min. Although this represented a significant increase ($P < 0.05$) over the nadir, it was not significantly greater than the basal rate of glucose production. Administration of glucagon to patient nos. 4, 5, and 6 led to a prompt increase in the rate of hepatic glucose production.

Table 2 contains a comparison of the basal and peak levels of glucagon, epinephrine, norepinephrine, growth hormone, and cortisol during insulin-induced hypoglycemia. There was a significant increase above baseline in all hormones except glucagon, whose levels did not increase significantly despite the stimulus of profound hypoglycemia. Only in patient no. 5 was a measurable increase in peripheral glucagon detected (Figure 1).

DISCUSSION

The success that has recently been achieved[2,22] in obtaining near-normal glucose levels in type I diabetics with continuous subcutaneous insulin infusion has provided an opportunity to determine which diabetic complications are reversed or delayed by optimal glycemic control. Blunted glucagon responses to hypoglycemia are commonly found in type I diabetics and it has been suggested that this abnormality may contribute to the development of severe hypoglycemia, which occurs in some of these patients.[23] The effect of long-term optimal control on counterregulatory glucagon responses has not been previously evaluated. In the present study hypoglycemia was induced by the acute administration of insulin in six patients who had been under optimal glucose control for 4–18 mo as evidenced by normal glycosylated hemoglobin and blood glucose values. Although plasma glucose fell to 27 ± 2.4 mg/dl during this test, plasma glucagon showed no increase in five of the six patients. In contrast to glucagon, plasma epinephrine, norepinephrine, growth hormone, and cortisol increased in each patient. The changes in the rate of hepatic glucose production that were documented in this study are also compatible with a deficiency in glucagon response. Thus, on stopping the insulin infusion, the Ra rose from a nadir of 0.5 ± 0.2 mg/kg/min to a peak of 2.5 ± 0.5 mg/kg/min. This peak value was not significantly different from baseline Ra. In a previous study using an experimental protocol similar to the present one, peak Ra values of 5.68 mg/kg/min were measured in healthy control subjects.[24] Furthermore, the adminstration of glucagon to three of the six patients in the present study resulted in a prompt increase in both plasma glucose levels and rate of glucose production. This finding excludes an intrinsic hepatic abnormality as an explanation for the blunted increase in glucose production in these patients.

The pathogenesis of the abnormal glucagon response to hypoglycemia in type I diabetics is unknown. Hyperglucagonemia and hyperresponsiveness of the hormone to arginine stimulation are also well recognized in this condition, but these abnormalities are corrected by insulin therapy,[25] suggesting that they are secondary to insulin deficiency. The present results indicate that this is not true of the blunted glucagon responses to hypoglycemia and suggest that these abnormal responses are due to a coexistent abnormality in the alpha cell, as has been suggested by Unger and Orci.[26] It is of interest that anti-alpha-cell antibodies have been described in type I diabetics,[27] but their potential role in causing the abnormalities of glucagon secretion requires further in-

TABLE 2
Levels of counterregulatory hormones measured during the baseline in comparison to peak levels reached when plasma glucose was <45 mg/dl

Hormone	Basal	Peak	P value
Glucagon (pg/ml)	104 ± 54.2	130.6 ± 84.0	NS
Epinephrine (pg/ml)	46.8 ± 13.3	477.2 ± 109.1	$P < 0.005$
Norepinephrine (pg/ml)	235.7 ± 49.4	483.5 ± 94.3	$P < 0.025$
Cortisol (ng/ml)	5.5 ± 0.7	16.7 ± 2.5	$P < 0.005$
Growth hormone (ng/dl)	9.3 ± 3.5	54.2 ± 11.7	$P < 0.005$

vestigation. Autonomic neuropathy[28] has also been implicated as a cause for the flat glucagon responses in type I diabetics. Although it is difficult to totally exclude mild subclinical autonomic neuropathy, none of our patients had clinically detectable autonomic neuropathy.

The effect of insulin pump therapy on the frequency and severity of hypoglycemic reactions is at present uncertain, although serious hypoglycemia has been reported in patients using this mode of insulin delivery.[29] In the present study only patient no. 1 had suffered a hypoglycemic episode serious enough to require medical assistance while the remaining patients only experienced symptoms compatible with mild hypoglycemia. Of greater concern is the fact that 7% of all home blood glucose values measured by these patients were <45 mg/dl and only 50% of these readings were associated with hypoglycemic symptoms. The effect of repeated episodes of asymptomatic insulin-induced hypoglycemia is not currently known.

The regulatory mechanisms protecting these patients from the development of more serious life-threatening hypoglycemic reactions are speculative. It has been suggested that epinephrine may play a critical role in the prevention of hypoglycemia in the absence of glucagon. All six patients did have significant epinephrine responses to the hypoglycemic stimulus. However, we have recently shown that the frequency and severity of clinical hypoglycemia do not always correlate with the abnormalities in counterregulatory hormones on objective testing.[24] A similar conclusion has been suggested by the data of Lager et al.[30] These workers showed that if plasma glucose levels were normalized by overnight insulin infusion, the counterregulatory increase in plasma glucose after insulin-induced hypoglycemia was enhanced. This improvement in plasma glucose recovery occurred despite reduced hormonal responses.

Unger[31] has recently warned of the possible danger of serious hypoglycemia that may occur in patients treated with aggressive regimens of insulin replacement. The results of the present study underscore the need for caution on the selection and treatment of patients with the insulin pump, since the blunted glucagon responses to hypoglycemia, one of the common metabolic abnormalities in type I diabetics, frequently persist despite long-term good glucose control. It is also evident that this glucagon unresponsiveness may be associated with only mild symptomatic hypoglycemia, although a high incidence of asymptomatic hypoglycemia appears to be present in these patients. Large-scale prospective studies are clearly necessary to define the risk of serious hypoglycemia in patients treated with aggressive regimens of insulin replacement, such as the insulin pump, and to assess the importance of disordered counterregulatory responses in determining this risk. Our data suggest, however, that abnormal glucagon responses to hypoglycemia are often present in patients treated with the insulin pump, and may be associated with a significant risk of hypoglycemia.

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REFERENCES

[1] Raskin, P., Pietri, A., and Unger, R.: Changes in glucagon levels after four to five weeks of glucoregulation by portable insulin infusion pumps. Diabetes 1979; 28:1033–35.

[2] Sherwin, R. S., Tamborlane, W. V., Genel, M., and Felig, P.: Treatment of juvenile-onset diabetics by subcutaneous infusion of insulin with a portable pump. Diabetes Care 1980; 3:301–308.

[3] Tamborlane, W. V., Sherwin, R. S., Genel, M., and Felig, P.: Restoration of normal lipid and aminoacid metabolism in diabetic patients treated with a portable insulin infusion pump. Lancet 1979; 1:1258–61.

[4] Gertner, J. M., Tamborlane, W. V., Horst, R. L., Sherwin, R. S., Felig, P., and Genel, M.: Mineral metabolism in diabetes mellitus changes accompanying treatment with a portable subcutaneous insulin infusion system. J. Clin. Endocrinol. Metab. 1980; 50:862–66.

[5] Gerich, J. E., Langlois, M., Naocco, C., Karam, J., and Forsham, P. H.: Lack of glucagon response to hypoglycemia in diabetes: evidence of an intrinsic pancreatic alpha cell defect. Science 1973; 182:171–73.

[6] Gerich, J., Davis, J., Lorenzi, M., et al.: Hormonal mechanisms of recovery from insulin-induced hypoglycemia in man. Am. J. Physiol. 1979; 5:E380–85.

[7] Bolli, G., Calabrese, G., DeFeo, P., Compagnucci, P., Zega, G., Angeletti, G., Cartechini, M. G., Santeusanio, F., and Brunetti, P.: Lack of glucagon response in glucose-counterregulation in type I (insulin-dependent) diabetics: absence of recovery after prolonged optimal insulin therapy. Diabetologia 1982; 22:100–105.

[8] Ensinck, J. W., and Kanter, R. A.: Glucagon responses to hypoglycemia in type I diabetic men after 24 hour glucoregulation by glucose controlled insulin infusion. Diabetes Care 1980; 3:285–89.

[9] Steno Study Group: Effect of 6 months of strict metabolic control on eye and kidney function in insulin dependent diabetics with background retinopathy. Lancet 1982; 1:121–23.

[10] Ewing, D. J., Campbell, I. W., and Clarke, B. F.: Assessment of cardiovascular effects in diabetic autonomic neuropathy and prognostic implications. Ann. Intern. Med. 1980; 92 (Part 2):308–11.

[11] Kuku, S. F., Jaspan, J. B., Emmanuouel, D. S., Zeidler, A., Katz, A. I., and Rubenstein, A. H.: Heterogeneity of plasma glucagon circulating components in normal subjects and patients with chronic renal failure. J. Clin. Invest. 1976; 58:742–50.

[12] Kuzuya, H., Blix, P. M., Horwitz, D. L., Steiner, D. F., and Rubenstein, A. H.: Determination of free and total insulin and C-peptide in insulin treated diabetics. Diabetes 1977; 26:22–29.

[13] Passon, P. G., and Peuler, J. D.: A simplified radiometric assay for norepinephrine and epinephrine. Anal. Biochem. 1973; 51:618–31.

[14] Odell, W. D., Rayford, P. L., and Ross, G. T.: Simplified partially automated method for radioimmunoassay of human thyroid stimulating, growth, luteinizing and follicle stimulating hormones. J. Lab. Clin. Med. 1967; 70:973–80.

[15] Murphy, B. E. P.: Some studies of the protein binding of steroids and their application to the routine micro and ultramicro measurement of various steroids in body fluids by competitive binding radioassay. J. Clin. Endocrinol. Metab. 1967; 27:973–90.

[16] Gabbay, K. H., Sosenko, J. M., Banuchi, G. A., Minisohn, M. H., and Fluckiger, R.: Glycosylated hemoglobins: increased glycosylation of hemoglobin A in diabetic patients. Diabetes 1979: 28:337–40.

[17] Wall, J. S., Steele, R., DeBodo, R. C., and Altszuler, N.: Effect of insulin on utilization and production of circulating glucose. Am. J. Physiol. 1957; 189:43–50.

[18] DeBodo, R. C., Steele, R., Altszuler, N., Dunn, A., and Bishop, J. S.: On the hormonal regulation of carbohydrate metabolism; studies with C^{14} glucose. Recent Prog. Horm. Res. 1963; 9:445–88.

[19] Somogyi, M.: Determination of blood sugar. J. Biol. Chem. 1945; 160:69–73.

[20] Radziuk, J., Norwich, K. H., and Vranic, M.: Measurement and validation of nonsteady turnover rates with applications to the insulin and glucose systems. Fed. Proc. 1974; 33:1855–64.

[21] Cherrington, A. D., and Vranic, M.: Effects of arginine on glucose turnover and plasma free fatty acids in normal dogs. Diabetes 1973; 22:537–43.

[22] Pickup, J. C., Keen, H., Viberti, G. C., et al.: Continuous subcutaneous insulin infusion in the treatment of diabetes mellitus. Diabetes Care 1980; 3:290–300.

[23] Boden, G., Reichard, G. A., Hoeldtke, R. D., Rezvani, I., and Owen, O. E.: Severe insulin induced hypoglycemia associated with deficiences in the release of counterregulatory hormones. N. Engl. J. Med. 1981; 305:1200–1205.

[24] Polonsky, K., Bergenstal, R., Pons, G., Schneider, M., Jaspan, J., and Rubenstein, A.: Relation of abnormal counterregulatory responses to hypoglycemia in type I diabetics. N. Engl. J. Med. 1982; 307:1106–12.

[25] Gerich, J. E., Tsalikian, E., Lorenzi, M., et al.: Normalization of fasting hyperglucagonemia and excessive glucagon responses to intravenous arginine in human diabetes mellitus by prolonged infusion of insulin. J. Clin. Endocrinol. Metab. 1976; 41:1178–80.

[26] Unger, R., and Orci, L.: Glucagon and the A cell: physiology and pathophysiology. N. Engl. J. Med. 1981; 304:1575–80.

[27] Van D. Winkel, M., Smets, G., Gepts, W., and Pipeleers, D.: Islet cell surface antibodies from insulin dependent diabetics bind specifically to pancreatic alpha cells. J. Clin. Invest. 1982; 70:41–49.

[28] Maher, T. D., Tanenberg, R. J., Greenberg, B. Z., Hoffman, J. E., Doe, R. P., and Goetz, F. C.: Lack of glucagon response to hypoglycemia in diabetic autonomic neuropathy. Diabetes 1977; 26:196–200.

[29] Lock, D. R., and Rigg, L. A.: Hypoglycemic coma associated with subcutaneous insulin infusion by portable pump. Diabetes Care 1981; 4:389–91.

[30] Lager, I., Blohmé, G., Von Schenck, H., and Smith, U.: Importance of glucose control for the recovery from hypoglycemia in insulin dependent diabetics. Diabetes 1982; 31:771–75.

[31] Unger, R. H.: Meticulous control of diabetes: benefits, risks and precautions. Diabetes 1982; 31:479–83.