Prevention of Hypoglycemia While Achieving Good Glycemic Control in Type 1 Diabetes

The role of insulin analogs

PAOLO ROSSETTI, MD
FRANCESCA PORCELLATI, MD, PHD
GEREMIA B. BOLLI, MD
CARMINE G. FANELLI, MD, PHD

Insulin therapy in diabetes, both at onset and after several years' duration, is primarily directed to maintain near-normoglycemia to prevent the onset and/or delay progression of long-term complications (1,2). However, it is important that regimens of insulin therapy are designed not only to aim at near-normalizing blood glucose, but also to minimize the risk of hypoglycemia. Subjects with type 1 diabetes continuously drift between hyperglycemia and hypoglycemia. If the former prevails, long-term complications are frequently expected (1). On the other hand, hypoglycemia is not only dangerous and unpleasant, but may over time lead to the syndrome of hypoglycemia unawareness (3).

This is relevant in type 1 diabetes but also in type 2 diabetes, since over time, many type 2 diabetic subjects develop progressive pancreatic β-cell dysfunction requiring insulin therapy. Because in subjects with advanced type 2 diabetes the neuroendocrine responses to hypoglycemia are as abnormal as in type 1 diabetic patients (4), insulin therapy may become responsible for frequent and/or severe hypoglycemia in type 2 diabetic patients as well.

The goal of minimizing the risk of hypoglycemia while achieving good glycemic control is feasible as long as *1*) a rational plan of insulin therapy is adopted, *2*) blood glucose is properly monitored, *3*) blood glucose targets are individualized, and *4*) education programs are widely implemented.

In the present article, the importance of the use of insulin analogs as a key tool to achieve good glycemic control and prevent hypoglycemia is emphasized.

PHYSIOLOGY OF PLASMA GLUCOSE HOMEOSTASIS

— Normal nondiabetic subjects maintain plasma glucose <100 mg/dl in the fasting and <135 mg/dl in the postprandial period. In the fasting state, this is due to the continuous release of insulin from the pancreas, which results in steady plasma insulin, thus restraining hepatic glucose production and thereby preventing fasting hyperglycemia. At mealtime, the normal pancreas releases insulin early in response to meal ingestion with a resultant acute plasma peak. This prevents postprandial hyperglycemia. Similarly important is the prompt decrease of plasma insulin 60–90 min after meal ingestion, which prevents hypoglycemia in the postprandial state. Finally, between-meal plasma insulin, especially during nocturnal fasting, is flat and peakless to prevent interprandial and fasting hypoglycemia (Fig. 1).

Nature's model of insulin dynamics should be mimicked whenever insulin is replaced in subjects with absent endogenous insulin secretion (type 1 diabetes). In the fasting state, insulin should be replaced with a preparation of "basal" insulin reproducing a flat peakless concentration (Fig. 1), since an insulin preparation that resulted in a peak would likely induce hypoglycemia in the fasting state. On the other hand, any insulin that would "wane" during fasting would result in hyperglycemia.

At mealtime, a bolus injection of rapid-acting insulin is needed to reproduce the early and high peak plasma insulin in concurrence with carbohydrate ingestion. Ideally, the faster the time-to-peak of the injected preparation, the lower the increase in postprandial hyperglycemia. After meals, plasma insulin should rapidly return to baseline; in fact, if plasma insulin remained elevated at the time at which carbohydrate absorption were completed, hypoglycemia would then develop.

DEFINITION OF HYPOGLYCEMIA

— Hypoglycemia is traditionally defined by *1*) the development of symptoms (autonomic or neuroglycopenic), *2*) a low plasma glucose level, and *3*) reversal of symptoms when the blood glucose level is restored to normal (Whipple's triad). Recently, the American Diabetes Association Workgroup on Hypoglycemia (5) agreed on how hypoglycemia must be defined and reported: the biochemical definition of hypoglycemia is a blood glucose concentration ≤70 mg/dl (3.9 mmol/l) (5). This definition is probably a conservative one but has the advantage of establishing safer glycemic targets during intensive insulin treatment, as initially proposed by Lalli et al. (6).

FREQUENCY OF HYPOGLYCEMIA

— Hypoglycemia is one of the more worrisome and distressing problems in diabetes. Depending on the severity of hypoglycemia,

From the Department of Internal Medicine, Section of Internal Medicine, Endocrinology and Metabolism, University of Perugia, Perugia, Italy.

Address correspondence and reprint requests to Prof. Geremia B. Bolli, University of Perugia, Department of Internal Medicine, Section of Internal Medicine, Endocrinology and Metabolism, Via E. Dal Pozzo, 6126 Perugia, Italy. E-mail: gbolli@unipg.it.

G.B.B. has received honoraria and consulting fees from sanofi-aventis, Novo Nordisk, Eli Lilly, Mannkind, Pfizer, Roche Pharmaceuticals, and Bioton. P.R., F.P., and C.G.F. have no duality of interest to disclose.

This article is based on a presentation at the 1st World Congress of Controversies in Diabetes, Obesity and Hypertension (CODHy). The Congress and the publication of this article were made possible by unrestricted educational grants from MSD, Roche, sanofi-aventis, Novo Nordisk, Medtronic, LifeScan, World Wide, Eli Lilly, Keryx, Abbott, Novartis, Pfizer, Generx Biotechnology, Schering, and Johnson & Johnson.

Abbreviations: CSII, continuous subcutaneous insulin infusion; MDI, multiple daily injections.

DOI: 10.2337/dc08-s227

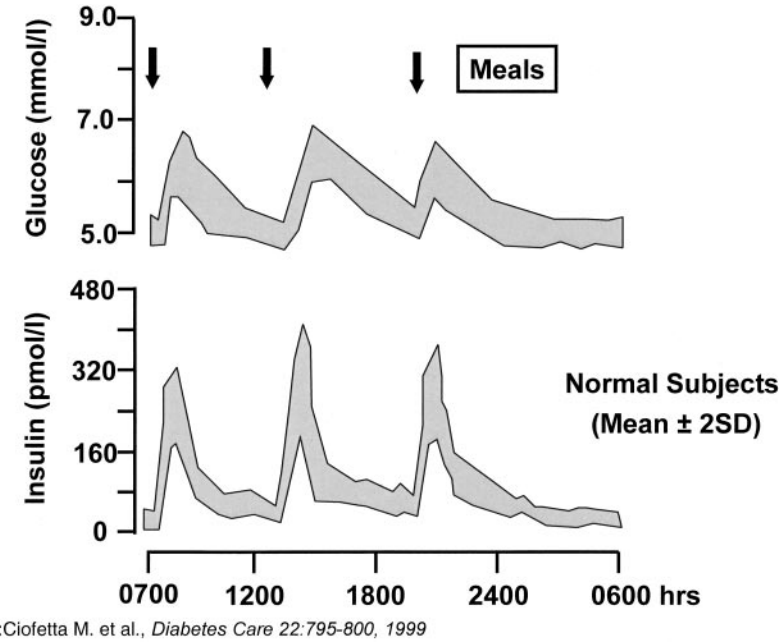


Figure 1—*Physiology of glucose homeostasis in normal nondiabetic subjects (adapted with permission).*

it can remarkably compromise and interfere with the intellectual and physical activities of the patient. In addition, hypoglycemia can generate psychological consequences (e.g., stress, negative moods, anxiety, irritability, family and work relationship problems, social isolation, etc.). For that reason, hypoglycemia is not a pleasant experience for patients and is feared. In many cases, it is a factor limiting the achievement of good glycemic control and can contribute to morbidity and mortality associated with diabetes.

In the real life of patients on intensive insulin treatment, hypoglycemia is quite common. However, data on the frequency of hypoglycemia in diabetic subjects are uncertain. While there is much more information on the frequency of severe hypoglycemia, little is known on the real frequency of asymptomatic and mild hypoglycemia. In fact, episodes of mild hypoglycemia (treated by patients themselves, often ignored, especially at night) are underestimated and underreported. However, mild hypoglycemia may exert profound negative effects in diabetic subjects, especially if recurrent, such as unawareness of hypoglycemia and impaired glucose counterregulation. The frequency of mild hypoglycemia may be estimated in the order of 0.1–0.3 episodes/patient-day (7). In the Diabetes Control and Complications Trial, the frequency of severe hypoglycemia increased threefold in diabetic subjects treated with intensive insulin therapy compared with subjects on conventional therapy (~0.6 vs. ~0.2 episodes/patient-year) (1). Similarly, in the Stockholm Diabetes Intervention Study, severe hypoglycemia was 2.5 times greater in the intensively treated patients (2). In type 2 diabetes, the rates of severe hypoglycemia are lower than in type 1 diabetes. This is not surprising because type 2 diabetes is characterized by insulin resistance, persistent β-cell function (which allows endogenous insulin secretion to decrease as blood glucose falls), and reduced glucagon response, at least in an early phase (8–10). The Kumamoto study (Japanese nonobese type 2 diabetic subjects) did not report episodes of severe hypoglycemia over 8-year observations (11). In the VA Cooperative study (12) and U.K. Prospective Diabetes Study (13), the frequency of severe hypoglycemia was much lower than that reported in the Diabetes Control and Complications Trial. However, in the U.K. Prospective Diabetes Study, the frequency of hypoglycemia increased over the years, along with duration of insulin treatment (14). Recent data provide further evidence that the risk of hypoglycemia in insulin-treated type 2 diabetes rises with increasing duration of insulin therapy (15). In another study, the incidence of severe hypoglycemia in type 2 diabetic patients was found to be as high as in type 1 diabetic patients after matching for duration of insulin therapy (16). Taken together, these data indicate that the risk of hypoglycemia increases in insulin-treated type 2 diabetic patients of long duration. Subjects with long-term type 2 diabetes on insulin treatment have nearly absent C-peptide concentration, and therefore reduced glucagon responses, as well as lower sympathoadrenal responses to hypoglycemia (4). Therefore, the important, modern concept here is that subjects with type 2 diabetes should be looked at as people at risk for hypoglycemia similarly to type 1 diabetic patients, especially when duration of type 2 diabetes is several years and they have long been treated with insulin.

NORMAL RESPONSES TO HYPOGLYCEMIA AND THEIR PATHOPHYSIOLOGY IN DIABETES

— From a practical point of view, the brain is totally dependent on a continuous supply of glucose from arterial circulation for its metabolism and function. It is glucose oxidation that normally provides most of the energy needed for brain function. In addition, the brain cannot synthesize glucose, nor does it have reserves (glycogen), except for a few minutes. As a consequence, the brain is the organ that is most exposed to the adverse effects of hypoglycemia.

In the clinical setting, hypoglycemia is the result of both iatrogenic hyperinsulinemia, which can be considered the initiating cause of hypoglycemia, and defective glucose counterregulation, which is responsible for the severity of hypoglycemia. In fact, because of the therapeutic delivery of insulin into the peripheral circulation, after subcutaneous administration, rather than into portal circulation, hyperinsulinemia is the rule in diabetes. However, at matched hyperinsulinemia, hypoglycemia is more severe and prolonged in subjects with type 1 diabetes than nondiabetic patients (17), indicating impaired defenses against hypoglycemia (17).

Normally, the protective responses to hypoglycemia are driven by cerebral glucose sensors (18). These responses include, first, release of counterregulatory hormones (19) and, second, generation of specific symptoms. In the hierarchy of counterregulation (20), the first mechanism is suppression of endogenous insulin secretion, which limits portal hyperinsulinemia (21). The second mechanism is an increase in all counterregulatory hormones. In fact, several studies have indicated that during insulin-

induced hypoglycemia, the sequence of hormonal counterregulatory responses is as follows: *1*) suppression of insulin release (occurring at a threshold glucose concentration of ~78–80 mg/dl), *2*) all counterregulatory hormones (glucagon, adrenaline, cortisol, growth hormone) are released at a (arterial) plasma glucose threshold of ~65 mg/dl (~3.5 mmol/l), *3*) symptoms (both autonomic and neuroglycopenic) appear only when plasma glucose decreases to ~55 mg/dl (~3.0 mmol/l), and *4*) cognitive function deteriorates when plasma glucose reaches ~50–54 mg/dl (19,20,22). In addition, it is possible that a greater availability of substrates (e.g., free fatty acids) contributes, at least in part, to the counterregulation of hypoglycemia (23). Symptoms include the autonomic (anxiety, palpitations, hunger, sweating, irritability, tremor) and neuroglycopenic (dizziness, tingling, blurred vision, difficulty in thinking, faintness) symptoms (19).

An important concept is that the brain responses to hypoglycemia are hierarchic (19). Over the last decade, a new concept regarding glucose thresholds for initiation of protective responses to hypoglycemia has gained ground. These glucose thresholds are not fixed, but rather are dynamic and are affected by antecedent prevailing ambient plasma glucose. Glucose thresholds shift downward (i.e., at lower plasma glucose concentrations) after hypoglycemia, either recurrent or chronic (24–32). As a consequence, responses to hypoglycemia require plasma glucose to reach levels lower than normal. This increases the risk of brain dysfunction and severe hypoglycemia (33). The importance of this observation relies on the fact that patients may or may not experience symptoms of hypoglycemia, depending on their recent antecedent blood glucose control. For example, if patients had had recurrent (i.e., daily) episodes of hypoglycemia, it is likely that they would not be able to recognize hypoglycemia at all, or possibly become aware of it at lower than normal plasma glucose concentrations.

It is of clinical relevance that, generally, patients rely mostly on the perception of the autonomic symptoms to recognize hypoglycemia even when neuroglycopenic symptoms are present. However, since perception of the autonomic symptoms is largely variable in relationship to antecedent glycemic control (e.g., symptoms reduced or absent in unaware subjects), more importance should be given to the recognition of neuroglycopenic symptoms for the early perception and correction of hypoglycemia.

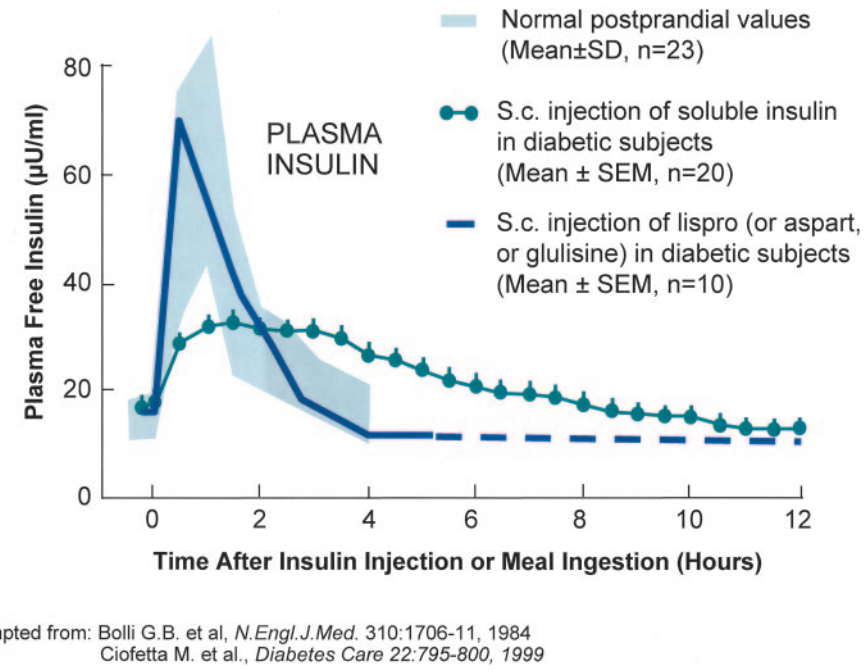
The most common counterregulatory defect in type 1 diabetes is loss of glucagon response to hypoglycemia, which progresses with progressive β-cell failure, supporting a permissive role of pancreatic β-cell on α-cell function during hypoglycemia (17). Under these conditions, it is the response of adrenaline that remains critical for counterregulation (17). Unfortunately, many type 1 diabetic subjects also suffer from reduced responses of adrenaline, especially after frequent recurrent hypoglycemia (34) and/or after many years of duration of type 1 diabetes (35,36). It is especially the type 1 diabetic subjects with combined defects of glucagon and adrenaline who are at high risk for severe hypoglycemia during insulin therapy, particularly if aiming at near-normoglycemia. As stated earlier, hypoglycemia is in general less frequent in type 2 diabetes than in type 1 diabetes, but it may become similarly frequent with longer duration of diabetes and insulin treatment, when the defects in counterregulation in type 2 diabetes (i.e., advanced failure to secrete both insulin and glucagon in response to hypoglycemia) approach those of type 1 diabetes. In addition, antecedent hypoglycemia reduces autonomic and symptomatic responses to subsequent hypoglycemia in type 2 diabetes as it does in type 1 diabetes (3).

ANTECEDENT HYPOGLYCEMIA AS A PRIMARY CAUSE OF HYPOGLYCEMIA UNAWARENESS IN DIABETES

— Over the last few years, there has been growing evidence that loss of symptoms of hypoglycemia in type 1 diabetic subjects, a condition named hypoglycemia unawareness, is largely, if not fully, the result of antecedent frequent hypoglycemia. The observation in insulinoma patients (37) that the surgical cure of hypoglycemia (resection of the tumor) is followed by full recovery of appropriate responses of counterregulatory hormones (of symptoms as well as onset of cognitive dysfunction) has prompted similar observations in type 1 diabetes. Fanelli et al. (34) were the first to report that meticulous prevention of hypoglycemia in type 1 diabetic subjects previously experiencing nearly one episode of hypoglycemia per day is followed by rapid recovery of symptoms (both autonomic and neuroglycopenic) and counterregulatory responses. In that study, symptoms were normalized both in terms of plasma glucose thresholds as well as magnitude (34). The release of counterregulatory hormones improved, particularly for adrenaline. However, the positive effect of prevention of hypoglycemia on responses of adrenaline is more evident in diabetes of short compared to long duration (35) and in diabetes without compared to with clinically overt autonomic neuropathy (36). The fact that some responses of adrenaline to hypoglycemia are apparently irreversibly lost, despite meticulous prevention of hypoglycemia in diabetes, especially in diabetes of long duration in the absence of autonomic neuropathy (25,35), cannot be easily explained at the present time. However, these data (25,35) indicate that loss of adrenaline response to hypoglycemia is not necessarily the result of autonomic neuropathy, although autonomic neuropathy importantly contributes to this finding (36). These results have subsequently been confirmed (25,38,39). Finally, induction of experimentally controlled hypoglycemia in normal human volunteers during the day (30), or the night (26,27), blunts the hormonal and symptom responses to hypoglycemia induced on the following day. Similar observations have been made in type 1 diabetes (28,31). When considering these data, it is evident that frequent hypoglycemia in type 1 diabetic patients rapidly induces loss of symptoms (unawareness) and blunts the release of counterregulatory hormones in response to hypoglycemia, exposing them to a greater risk for severe hypoglycemia (40).

Mechanisms of hypoglycemia unawareness

There is evidence that rates of glucose transport from the blood to the brain may be affected by prevailing antecedent glucose concentration (41,42). Indeed, the transport of circulating glucose into cerebral cells is an insulin-independent process that requires the presence of facilitative glucose transport proteins (43). Recent studies indicate that the expression of the glucose transporters GLUT1 (55-kDa form, localized in microvessels of the blood-brain barrier) (44) and GLUT3 (the neuron-specific glucose transporter) proteins is increased after chronic insulin-induced hypoglycemia in rats (45).



Adapted from: Bolli G.B. et al, *N.Engl.J.Med.* 310:1706-11, 1984
Ciofetta M. et al., *Diabetes Care 22:795-800, 1999*

Figure 2—*Pharmacokinetics after subcutaneous injection of regular unmodified human insulin and rapid-acting insulin analogs (adapted with permission).*

The upregulation of glucose transporter expression described in animals may be relevant to the clinical phenomenon of hypoglycemia unawareness in humans (34), which appears to be mediated by similar mechanisms (46). These findings suggest that antecedent hypoglycemia accelerates delivery of glucose to the brain, which then is "less" neuroglycopenic than normal and does not need to generate the responses to subsequent hypoglycemia. However, recent positron emission tomography technique–based studies have not confirmed the above conclusions in healthy subjects after antecedent hypoglycemia (47) or in chronically hyperglycemic subjects with type 1 diabetes (48), although the occurrence of regional cerebral changes in these studies cannot be excluded. Finally, reduced global neuronal activation has been documented in unaware compared with aware diabetic subjects (49).

An additional mechanism of hypoglycemia unawareness has been provided by Davis et al. (50). They found that the responses of cortisol to antecedent hypoglycemia blunt the autonomic hormone responses to subsequent hypoglycemia (50). However, recently it has been shown that cortisol elevation, comparable to that occurring during hypoglycemia, did not reduce neuroendocrine responses to subsequent hypoglycemia (51,52). Finally, the application of urocortin I, a corticotrophin-releasing factor receptor-2 agonist, in the ventromedial hypothalamus suppresses the counterregulatory response to hypoglycemia (53). The above observations indicate that the mechanisms of hypoglycemia unawareness, including the role of cortisol, are not yet well understood. Most likely, multiple mechanisms combine to generate this syndrome.

In the last few years, research has focused on the role of substrates other than glucose, such as lactate, and brain glycogen metabolism and on their role in sustaining brain function under conditions of energy depletion (neuroglycopenia). There is growing in vitro and in vivo evidence that lactate can support neuron metabolism in different circumstances (54) involving neuronal activation and under conditions of energy crisis (55). Interestingly, most of the lactate used by neurons is formed within the brain rather than being borne by blood (56). According to the hypothesis suggested by Pellerin et al. (57,58), lactate produced by astrocytes would fuel neurons by an astrocyte-neuron lactate shuttle mechanism. However, the extent to which lactate can substitute for glucose in sustaining neuronal activity and function has not been completely established (59,60). The function of brain glycogen is not well understood. Although overall brain glycogen content is low (~3 µmol/g) (61,62), it has been suggested that even under normal conditions, cerebral glycogen is important for brain function (62).

THE VICIOUS CIRCLE OF RECURRENT HYPOGLYCEMIA UNAWARENESS

— Hypoglycemia occurs unavoidably from time to time in diabetic subjects whose glycemic target is near-normoglycemia in the setting of deficient glucagon response to hypoglycemia and impaired secretion of adrenaline (17). If hypoglycemia is frequent and recurrent, it causes the initial loss of the warning symptoms and, over time, hypoglycemia unawareness. Impaired adrenaline secretion (and glucagon secretion) and hypoglycemia unawareness, a condition known as hypoglycemia-associated autonomic failure, in turn foster both recurrent and severe hypoglycemia (63,64). Therefore, prevention of hypoglycemia is an important part of modern intensive diabetes therapy (65). In fact, meticulous prevention of hypoglycemia in diabetic subjects suffering from unawareness fully reverses the syndrome of hypoglycemia unawareness and impaired release of adrenaline in short-term diabetes (34) and improves responses in long-term diabetes (36).

BENEFITS OF INSULIN ANALOGS VERSUS HUMAN NONMODIFIED INSULIN

Rapid-acting insulin analogs

Lispro, aspart, and glulisine are different rapid-acting insulin analogs (66,67) that share similar pharmacokinetic and pharmacodynamic properties compared with human regular insulin (Fig. 2). Upon subcutaneous injection, these rapid-acting analogs reach the status of monomeric insulin earlier than human regular insulin and are therefore absorbed faster. The earlier and greater plasma insulin peak achieved with the analogs controls postprandial plasma glucose better than human regular insulin.

The advantages of the rapid-acting analogs can be summarized as follows. First, it is almost possible to mimic nature in terms of peak prandial insulin; therefore, it is possible to reduce postprandial hyperglycemia versus human regular insulin. Second, because of the early waning, insulin analogs reduce the risk of

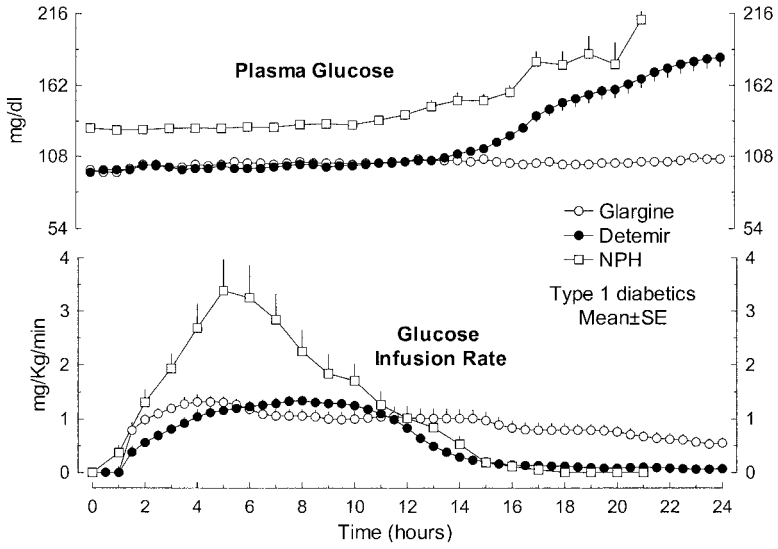


Figure 3—*Comparison of pharmacodynamics after subcutaneous injection of NPH, glargine, and detemir in type 1 diabetes (adapted with permission).*

postprandial hypoglycemia versus human regular insulin. Third, and perhaps the most important, insulin analogs improve quality of life, since subjects with type 1 diabetes can now "inject and eat." This is a great advantage when compared with earlier treatment, when subjects had to wait 15, 30, or 45 min between injection and meal ingestion.

At present, the rapid-acting analogs are the gold standard of mealtime insulin replacement in type 1 diabetes. They should substitute human regular insulin in all diabetic subjects, provided they are combined with optimal replacement of basal insulin (see below). Under these conditions, rapid-acting analogs lower A1C and reduce the risk of hypoglycemia (3). The former goal reduces risk for long-term complications (1), and the latter improves awareness of hypoglycemia (2).

Long-acting insulin analogs

In 1946, the Hagedorn's invented NPH insulin reached the market. Ever since then, NPH has been the bestseller of the "basal" insulin market. When NPH is analyzed with the glucose clamp technique (68) (Fig. 3), it does not mimic the flat peakless basal insulin of physiology (Fig. 1). NPH has a peak 5–6 h after injection and wanes a few hours later (Fig. 3). Thus, when injected in the evening, the peak action of NPH increases the risk of hypoglycemia right after midnight. On the other hand, the relatively short duration of action of NPH makes it very difficult to achieve near-normoglycemia in the fasting state without increasing risk for nocturnal hypoglycemia. Finally, since NPH is an insoluble preparation that needs to be resuspended before subcutaneous injection, NPH has an absorption that is quite variable, resulting in different fasting blood glucose from day to day. These are the reasons why NPH should no longer be used in type 1 diabetes. Conversely, the long-acting insulin analogs (glargine, detemir) or the technique of continuous subcutaneous insulin infusion (CSII) generate an optimal basal pharmacokinetic/pharmacodynamic profile and, for that reason, should be used to replace basal insulin in type 1 diabetes.

Glargine is a soluble long-acting insulin analog, peakless compared with NPH, with duration of action of 24 h and more (69). Since it is soluble, it is by definition more reproducible than NPH (70). The more physiological pharmacokinetics/pharmacodynamics of insulin glargine versus NPH translate into the clinical advantage of lower risk for nocturnal hypoglycemia with similar or lower A1C (3). Glargine should be given as a once-daily evening injection (either before or after dinner). The elevation in predinner blood glucose is not explained by duration of action of glargine <24 h, since glargine generally has duration of action >24 h (Fig. 3) (71). This is likely caused by delayed absorption of the lunch meal beyond end of action of rapid-acting insulin. Therefore, administration of twice-daily glargine is not justified, and may cause hypoglycemia from overlapping doses, although it has been adopted in a small group of type 1 patients (72). Rather, a dual bolus of rapid-acting insulin analog given at lunch (before and 3 h after) should optimize late-afternoon blood glucose.

Detemir also is a soluble long-acting insulin analog more reproducible than NPH (70). When compared with glargine, detemir is similarly peakless, but exhibits a shorter duration of action (Fig. 3) (71). Thus, in the majority of subjects with type 1 diabetes, detemir should be given every 12 h. A peculiar characteristic of detemir, not shared by NPH or by glargine, is that its long-term use is associated with less weight gain (0.5–1.5 kg) compared with the other basal insulin.

REGIMENS OF MULTIPLE DAILY INJECTIONS AND CSII

— In the NPH era, CSII has been shown to be superior to multiple daily injections (MDI) because the basal insulin delivered by CSII is soluble, whereas that of MDI was NPH (insoluble and therefore more variable) (70). In the era of soluble long-acting analogs, MDI is no longer inferior to CSII in terms of A1C and frequency of hypoglycemia (73–76). CSII has the theoretical advantage of lower variability compared with MDI, but so far this has been difficult to prove. Thus, the choice between MDI and CSII is based on preference by the individual type 1 diabetic subject rather than a real indication.

CONCLUSIONS

— When combined with appropriate education and motivation of the subjects with type 1 diabetes, insulin regimens based on insulin analogs (either MDI or CSII) successfully reach the glycemic targets of the Diabetes Control and Complications Trial, thus protecting against the risk of onset of long-term complications, and at the same time they minimize the frequency of hypoglycemia, prevent hypoglycemia unawareness, and improve quality of life.

Acknowledgments— This article is dedicated to the people with diabetes who have volunteered for our studies.

References

1. Diabetes Control and Complications Trial Research Group: The effect of intensive treatment of diabetes on the development and progression of long-term complications in insulin-dependent diabetes mellitus. *N Engl J Med* 329:977–986, 1993
2. Reichard P, Nilsson B-Y, Rosenquist U: The effect of intensive treatment of diabe-

tes on the development and progression of long-term complications in insulin-dependent diabetes mellitus. *N Engl J Med* 329:304–309, 1993
3. Bolli GB: Treatment and prevention of hypoglycemia and its unawareness in type 1 diabetes mellitus. *Rev Endocr Metab Dis* 4:335–341, 2003
4. Segel SA, Paramore DS, Cryer PE: Hypoglycemia-associated autonomic failure in advanced type 2 diabetes. *Diabetes* 51: 724–733, 2002
5. Workgroup on Hypoglycemia, American Diabetes Association: Defining and reporting hypoglycemia in diabetes: a report from the American Diabetes Association Workgroup on Hypoglycemia. *Diabetes Care* 28:1245–1249, 2005
6. Lalli C, Ciofetta M, Del Sindaco P, Torlone E, Pampanelli S, Compagnucci P, Cartechini MG, Bartocci L, Brunetti P, Bolli GB: Long-term intensive treatment of type 1 diabetes with the short-acting insulin analog lispro in variable combination with NPH insulin at mealtime. *Diabetes Care* 22:468–477, 1999
7. Fanelli CG, Pampanelli S, Porcellati F, Rossetti P, Brunetti P, Bolli GB: Administration of neutral protamine Hagedorn insulin at bedtime versus with dinner in type 1 diabetes mellitus to avoid nocturnal hypoglycemia and improve control: a randomized, controlled trial. *Ann Intern Med* 136:504–514, 2002
8. Cryer PE, Davis SN, Shamoon HS: Hypoglycemia in diabetes. *Diabetes Care* 26: 1902–1912, 2003
9. Spyer G, Hattersley A, MacDonald I, Amiel S, MacLeod H: Hypoglycaemic counter-regulation at normal blood glucose concentrations in patients with well controlled type-2 diabetes. *Lancet* 356: 1970–1974, 2000
10. Gerich J: Hypoglycemia and counter regulation in type 2 diabetes. *Lancet* 356: 1946–1947, 2000
11. Shichiri M, Kishikawa H, Ohkubo Y, Wake N: Long term results of the Kumamoto study on optimal diabetes control in type 2 diabetic patients. *Diabetes Care* 23:B21–B29, 2000
12. Abraira C, Henderson WG, Colwell JA, Nuttall FQ, Comstock JP, Emanuele NV, Levin SR, Sawin CT, Silbert CK: Response to intensive therapy steps and to glipizide dose in combination with insulin in type 2 diabetes: VA feasibility study on glycemic control and complications (VA CSDM). *Diabetes Care* 21:574–579, 1998
13. UKPDS Research Group: Overview of 6 years of therapy of type II diabetes: a progressive disease. *Diabetes* 44:1249–1258, 1995
14. UKPDS Research Group: Effort of intensive blood glucose control with insulin and sulfonylureas on insulin compared with conventional treatment and risk of complications in patients with type 2 diabetes (UKPDS 33). *Lancet* 352:837–853, 1998
15. UK Hypoglycemia Study Group: Risk of hypoglycemia in types 1 and 2 diabetes: effects of treatment modalities and their duration. *Diabetologia* 50:1140–1147, 2007
16. Hepburn DA, MacLeod KM, Pell ACH, Scougal IJ, Frier BM: Frequency and symptoms of hypoglycemia experienced by patients with type 2 diabetes treated with insulin. *Diabet Med* 10:231–237, 1993
17. Bolli GB: From physiology of glucose counterregulation to prevention of hypoglycemia in type 1 diabetes mellitus. *Diab Nutr Metab* 4:333–349, 1990
18. Borg WP, Sherwin RS, During MJ, Borg MA, Shulman GI: Local ventromedial hypothalamus glucopenia triggers counterregulatory hormone release. *Diabetes* 44: 180–184, 1995
19. Mitrakou A, Ryan C, Veneman T, Mokan M, Jenssen T, Kiss I, Durrant J, Cryer P, Gerich J: Hierarchy of glycemic thresholds for counterregulatory hormone secretion, symptoms, and cerebral dysfunction. *Am J Physiol* 260:E67–E74, 1991
20. Fanelli C, Pampanelli S, Epifano L, Rambotti AM, Ciofetta M, Modarelli F, Di Vincenzo A, Annibale B, Lepore M, Lalli C, Del Sindaco P, Brunetti P, Bolli GB: Relative roles of insulin and hypoglycemia on induction of neuroendocrine responses to, symptoms of, and deterioration of cognitive function in hypoglycemia in male and female humans. *Diabetologia* 37:797–807, 1994
21. De Feo P, Perriello G, De Cosmo S, Ventura MM, Campbell PJ, Brunetti P, Gerich JE, Bolli GB: Comparison of glucose counterregulation during short-term and prolonged hypoglycemia in normal humans. *Diabetes* 35:563–569, 1986
22. Schwartz N, Clutter W, Shah S, Cryer P: Glycemic thresholds for activation of glucose counterregulatory systems are higher than the thresholds for symptoms. *J Clin Invest* 79:777–781, 1987
23. Fanelli C, Calderone S, Epifano L, De Vincenzo A, Modarelli F, Pampanelli S, Perriello G, De Feo P, Brunetti P, Gerich JE, Bolli GB: Demonstration of a critical role for free fatty acids in mediating counterregulatory stimulation of gluconeogenesis and suppression of glucose utilization in humans. *J Clin Invest* 92:1617–1622, 1993
24. Fruehwald-Schultes B, Born J, Kern W, Peters A, Fehm HL: Adaptation of cognitive function to hypoglycemia in healthy men. *Diabetes Care* 23:1059–1066, 2000
25. Fanelli C, Pampanelli S, Epifano L, Rambotti AM, Di Vincenzo A, Modarelli F, Ciofetta M, Lepore M, Annibale B, Torlone E, Perriello G, De Feo P, Santeusanio F, Brunetti P, Bolli GB: Long-term recovery from unawareness, deficient counterregulation and lack of cognitive dysfunction during hypoglycemia, following institution of a rational, intensive insulin therapy in IDDM. *Diabetologia* 37:1265–1276, 1994
26. Veneman T, Mitrakou A, Mokan M, Cryer P, Gerich J: Induction of hypoglycemia unawareness by asymptomatic nocturnal hypoglycemia. *Diabetes* 42:1233–1237, 1993
27. Hvidberg A, Fanelli CG, Hersbey T, Cryer PE: Impact of recent antecedent hypoglycemia on hypoglycemic cognitive dysfunction in nondiabetic humans. *Diabetes* 45:1030–1036, 1996
28. Fanelli CG, Paramore DS, Hershey T, Terkamp C, Ovalle F, Craft S, Cryer PE: Impact of nocturnal hypoglycemia on hypoglycemic cognitive dysfunction in type 1 diabetes. *Diabetes* 47:1920–1927, 1998
29. Ovalle F, Fanelli CG, Paramore DS, Hershey T, Craft S, Cryer PE: Brief twice-weekly episodes of hypoglycemia reduce detection of clinical hypoglycemia in type 1 diabetes mellitus. *Diabetes* 47:1472–1479, 1998
30. Heller S, Cryer PE: Reduced neuroendocrine and symptomatic responses to subsequent hypoglycemia after one episodes of hypoglycemia in nondiabetic humans. *Diabetes* 40:223–226, 1991
31. Dagogo-Jack SE, Cryer PE: Hypoglycemia-associated autonomic failure in insulin-dependent diabetes mellitus: recent antecedent hypoglycemia reduces autonomic responses to, symptoms of, and defense against subsequent hypoglycemia. *J Clin Invest* 91:819–828, 1993
32. Maran A, Lomas J, Macdonald IA, Amiel SA: Lack of preservation of higher brain function during hypoglycemia in patients with intensively treated IDDM. *Diabetologia* 38:1412–1418, 1995
33. Fanelli CG, Pampanelli S, Porcellati F: Cognitive function in hypoglycemia. *Diabetes Nutr Metab* 15:367–372, 2002
34. Fanelli CG, Epifano L, Rambotti AM, Pampanelli S, Di Vincenzo A, Modarelli F, Lepore M, Annibale B, Ciofetta M, Bottini P, Porcellati F, Scionti L, Santeusanio F, Brunetti P, Bolli GB: Meticulous prevention of hypoglycemia normalizes the glycemic thresholds and magnitude of most of neuroendocrine responses to, symptoms of, and cognitive function during hypoglycemia in intensively treated patients with short-term IDDM. *Diabetes* 42:1683–1689, 1993
35. Cranston I, Lomas J, Maran A, Macdonald I, Amiel SA: Restoration of hypoglycemia awareness in patients with long-duration insulin-dependent diabetes. *Lancet* 344: 283–287, 1994
36. Fanelli C, Pampanelli S, Lalli C, Del Sindaco P, Ciofetta M, Lepore M, Porcellati F, Bottini P, Di Vincenzo A, Brunetti P, Bolli

GB: Long-term intensive therapy of IDDM diabetic patients with clinically overt autonomic neuropathy: effects on awareness of, and counterregulation to hypoglycemia. *Diabetes* 46:1172–1181, 1997
37. Mitrakou A, Fanelli C, Veneman T, Perriello G, Calderone S, Platanisiotis D, Rambotti A, Raptis S, Brunetti P, Cryer P, Gerich PE, Bolli GB: Reversibility of unawareness of hypoglycemia in patients with insulinomas. *N Engl J Med* 329:834–839, 1993
38. Dagogo-Jack S, Rattarasarn C, Cryer PE: Reversal of hypoglycemia unawareness, but not defective glucose counter regulation, in IDDM. *Diabetes* 43:1426–1434, 1994
39. Liu D, McManus RM, Ryan EA: Improved counter-regulatory hormonal and symptomatic responses to hypoglycemia in patients with insulin-dependent diabetes mellitus after 3 months of less strict glycemic control. *Clin Invest Med* 19:71–82, 1996
40. Gold AE, MacLeod KM, Frier BM: Frequency of severe hypoglycemia in patients with type 1 diabetes with impaired awareness of hypoglycemia. *Diabetes Care* 17:697–703, 1994
41. McCall A, Fixman L, Fleming N, Tornheim K, Click W, Ruderman NB: Chronic hypoglycemia increases brain glucose transport. *Am J Physiol* 251:E442–E447, 1986
42. McCall AL, Millington WR, Wurtman RJ: Metabolic fuel and amino acid transport into the brain in experimental diabetes mellitus. *Proc Natl Acad Sci U S A* 79: 5406–5410, 1982
43. Vannucci SJ, Maher F, Simpson IA: Glucose transporter proteins in brain: delivery of glucose to neurons and glia. *Glia* 21:2–21, 1997
44. McCall AL, Fixman LB, Fleming N, Fleming L, Click W, Ruderman NB: Chronic hypoglycemia increases brain glucose transport. *Am J Physiol* 251:E442–E447, 1986
45. Uehara Y, Nipper V, McCall AL: Chronic insulin hypoglycemia induces GLUT-3 protein in rat brain neurons. *Am J Physiol* 272:E716–E719, 1997
46. Boyle PJ, Kempers SF, O'Connor AM, Nagy RJ: Brain glucose uptake and unawareness of hypoglycemia in patients with insulin dependent diabetes mellitus. *N Engl J Med* 333:1726–1731, 1995
47. Segel SA, Fanelli CG, Dence CS, Markham J, Videen TO, Paramore DS, Powers WJ, Cryer PE: Blood-to-brain glucose transport, cerebral glucose metabolism, and cerebral blood flow are not increased after hypoglycemia. *Diabetes* 50:1911–1917, 2001
48. Fanelli CG, Dence CS, Markham J, Videen TO, Paramore DS, Cryer PE, Powers WJ: Blood-to-brain glucose transport and cerebral glucose metabolism are not reduced in poorly controlled type 1 diabetes. *Diabetes* 47:1444–1450, 1998
49. Bingham EM, Dunn JT, Smith D, Sutcliffe-Goulden J, Reed LJ, Marsden PK, Amiel SA: Differential changes in brain glucose metabolism during hypoglycemia accompany loss of hypoglycemia awareness in men with type 1 diabetes mellitus: an [^{11}C]-3-*O*-methyl-D-glucose PET study. *Diabetologia* 48:2080–2089, 2005
50. Davis SN, Shavers C, Costa F, Mosqueda-Garcia R: Role of cortisol in the pathogenesis of deficient counterregulation after antecedent hypoglycemia in normal humans. *J Clin Invest* 98:680–691, 1996
51. Raju B, McGregor VP, Cryer PE: Cortisol elevations comparable to those that occur during hypoglycemia do not cause hypoglycemia-associated autonomic failure. *Diabetes* 52:2083–2089, 2003
52. Goldberg PA, Weiss R, McCrimmon RJ, Hintz EV, Dziura JD, Sherwin RS: Antecedent hypercortisolemia is not primarily responsible for generating hypoglycemia-associated autonomic failure. *Diabetes* 55: 1121–1126, 2006
53. McCrimmon RJ, Song Z, Cheng H, McNay EC, Weikart-Yeckel C, Fan X, Routh VH, Sherwin RS: Corticotrophin-releasing factor receptors within the ventromedial hypothalamus regulate hypoglycemia-induced hormonal counterregulation. *J Clin Invest* 116:1723–1730, 2006
54. Smith D, Pernet A, Hallett WA, Bingham E, Marsden PK, Amiel SA: Lactate: a preferred fuel for human brain metabolism in vivo. *J Cereb Blood Flow Metab* 23:658–664, 2003
55. Tsacopoulos M, Magistretti PJ: Metabolic coupling between glia and neurons. *J Neurosci* 16:877–885, 1996
56. Walz W, Murkerji S: Lactate production and release in cultured astrocytes. *Neurosci Lett* 86:296–300, 1988
57. Pellerin L, Pellegri G, Bittar PG, Charnay Y, Bouras C, Martin JL, Stella N, Magistretti PJ: Evidence supporting the existence of an activity-dependent astrocyte-neuron lactate shuttle. *Dev Neurosci* 20: 291–299, 1998
58. Pellerin L, Bonvento G, Chatton J-Y, Pierre K, Magistretti PJ: Role of neuron-glia interaction in the regulation of brain glucose utilization. *Diabetes Nutr Metab* 15:268–273, 2002
59. Chih C-P, Lipton P, Roberts EL: Do active cerebral neurons really use lactate rather than glucose? *Trends Neurosci* 24:573–578, 2001
60. Lubow JM, Pinon IG, Avogaro A, Cobelli C, Treeson DM, Mandeville KA, Toffolo G, Boyle PJ: Brain oxygen utilization is unchanged by hypoglycemia in normal humans: lactate, alanine, and leucine uptake are not sufficient to offset energy deficit. *Am J Physiol Endocrinol Metab* 290: E149–E153, 2006
61. Choi IY, Gruetter R: In vivo 13C NMR assessment of brain glycogen concentration and turnover in the awake rat. *Neurochem Int* 43:317–322, 2003
62. Clarke DD, Sokoloff L: Circulation and energy metabolism of the brain. In *Basic Neurochemistry: Molecular, Cellular and Medical Aspects.* 5th ed. Siegel G, Agranoff B, Albers RW, Molinoff P, eds. New York, Raven Press, 1994, p. 645–652
63. Cryer PE: Iatrogenic hypoglycemia as a cause of hypoglycemia-associated autonomic failure in IDDM: a vicious cycle. *Diabetes* 41:255–260, 1992
64. Cryer PE: Hypoglycemia-associated autonomic failure in diabetes. *Am J Physiol* 281:E1115–E1121, 2001
65. Pampanelli S, Fanelli C, Lalli C, Ciofetta M, Sindaco PD, Lepore M, Modarelli F, Rambotti AM, Epifano L, Di Vincenzo A, Bartocci L, Annibale B, Brunetti P, Bolli GB: Long-term intensive insulin therapy: effects of HbA1c risk for severe and mild hypoglycemia, status of counterregulation and unawareness of hypoglycemia. *Diabetologia* 39:677–686, 1996
66. Bolli GB, Di Marchi R, Park G, Pramming S, Koivisto VA: Insulin analogs and their potential in the management of diabetes mellitus. *Diabetologia* 42: 1151–1167, 1999
67. Becker RHA: Insulin glulisine complementing basal insulin: a review of structure and activity. *Diabetes Technol Ther* 9:109–121, 2007
68. Lepore M, Pampanelli S, Fanelli C, Porcellati F, Bartocci L, Di Vincenzo A, Cordoni C, Costa E, Brunetti P, Bolli GB: Pharmacokinetics and pharmacodynamics of the long-acting human insulin analog glargine, NPH insulin, and ultralente human insulin injected subcutaneously, and continuous subcutaneous infusion of insulin lispro. *Diabetes* 49:2142–2148, 2000
69. Porcellati F, Rossetti P, Busciantella Ricci N, Pampanelli S, Torlone E, Herdandez Campos S, Marinelli Andreoli A, Bolli GB, Fanelli CG: Pharmacokinetics and pharmacodynamics of the long-acting insulin analog glargine after one week of use as compared to its first administration in subjects with type 1 diabetes mellitus. *Diabetes Care* 30:1261–1263, 2007
70. Heise T, Nosek L, Ronn BB, Endahl L, Heinemann L, Kapitza C, Draeger E: Lower within-subject variability of insulin detemir in comparison to NPH insulin and insulin glargine in people with type 1 diabetes. *Diabetes* 53:1614–1620, 2004
71. Porcellati F, Rossetti P, Busciantella NR, Marzotti S, Lucidi P, Luzio S, Owens DR, Bolli GB, Fanelli CG: Comparison of pharmacokinetics and dynamics of the long-acting insulin analogs glargine and detemir at steady state in type 1 diabetes: a double-blind, randomized, crossover

study. *Diabetes Care* 30:2447–2452, 2007
72. Ashwell SG, Gebbie J, Home PD: Twice-daily compared with once-daily insulin glargine in people with type 1 diabetes using meal-time insulin aspart. *Diabet Med* 23:879–886, 2006
73. García-García E, Galera R, Aguilera P, Cara G, Bonillo A: Long-term use of continuous subcutaneous insulin infusion compared with multiple daily injections of glargine in pediatric patients. *J Pediatr Endocrinol Metab* 20:37–40, 2007
74. Hirsch IB, Bode BW, Garg S, Lane WS, Sussman A, Hu P, Santiago OM, Kolaczynski JW: Insulin Aspart CSII/MDI Comparison Study Group: Continuous subcutaneous insulin infusion (CSII) of insulin aspart versus multiple daily injection of insulin aspart/insulin glargine in type 1 diabetic patients previously treated with CSII. *Diabetes Care* 28:533–538, 2005
75. Herman WH, Ilag LL, Johnson SL, Martin CL, Sinding J, Al Harthi A, Plunkett CD, LaPorte FB, Burke R, Brown MB, Halter JB, Raskin P: A clinical trial of continuous subcutaneous insulin infusion versus multiple daily injections in older adults with type 2 diabetes. *Diabetes Care* 28: 1568–1573, 2005
76. Thomas RM, Aldibbiat A, Griffin W, Cox MA, Leech NJ, Shaw JA: A randomized pilot study in type 1 diabetes complicated by severe hypoglycemia, comparing rigorous hypoglycemia avoidance with insulin analog therapy, CSII or education alone. *Diabet Med* 24: 778–783, 2007