Physiological Responses to Hypoglycemia

Counterregulation and cognitive function

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Glucose is normally the exclusive fuel for cerebral metabolism, and blood glucose must be maintained above a critical level to preserve adequate brain function. Under normal circumstances, an exquisite homeostasis exists between glucose needs and glucose synthesis or mobilization from body stores. A cerebral glucose sensor, possibly located in the hypothalamus, has been postulated as the mechanism for recognition of declining blood glucose levels. Such low blood glucose (hypoglycemia) is common in people with IDDM. Properly functioning neurohumoral responses to hypoglycemia initiate physiological counterregulatory measures and alert the individual to the need for carbohydrate ingestion. These responses normally become operative at blood glucose levels exceeding those that cause significant cerebral dysfunction. Unfortunately, many diabetic patients, particularly those under tight metabolic control, have impaired physiological responses to hypoglycemia. Disregard of the signs of incipient hypoglycemia will exacerbate the condition and may lead to further blunting of normal physiological counterregulatory mechanisms. If the condition is not rectified promptly, concentration and intellectual performance will be impaired to the extent that the individual is no longer able to treat the condition alone. Although most of the effects of hypoglycemia abate when normal blood glucose levels are restored, some evidence points to the possibility of permanent sequelae when hypoglycemia is recurrent or chronic. Acutely, even moderate hypoglycemia can cause noticeable EEG changes. Thus, the ability to recognize the onset of hypoglycemia and institute appropriate countermeasures is of crucial importance to the diabetic patient.

IN HEALTHY HUMANS— Brain function depends on glucose that, under normal circumstances, is the exclusive fuel for cerebral metabolism. Because the brain can neither synthesize nor store glucose, a constant exogenous supply is essential (1,2). This is provided by the brain's blood supply. As blood glucose falls, a series of physiological responses occur that act to restore those levels. These responses also create symptoms that an individual can watch for, and that warn him or her to take corrective action by eating carbohydrates. If these counterregulatory responses fail and blood glucose falls below a critical level, cognitive function is impaired. Confusion and even coma may ensue.

The peripheral responses to hypoglycemia are, under normal circumstances, so efficient that clinically important hypoglycemia probably never occurs in health. In clinical practice, significant hypoglycemia is associated with relative hyperinsulinemia, and is commonly seen only in people taking exogenous hypoglycemic agents such as sulfonylureas or insulin. There is the rare patient with hyperinsulinemia that occurs secondary to tumors secreting insulin or insulinlike factors. Significant hypoglycemia very rarely may result from a deficit of insulin antagonists or from exhaustion of endogenous glucose supplies.

Insulin lowers blood glucose by limiting the release of glucose from endogenous stores, primarily the liver, and by stimulating uptake, oxidation, and storage of glucose by peripheral tissues, especially muscle and fat (3,4). Insulin antagonists, on the other hand, raise blood glucose by stimulating glucose synthesis and release from the liver, by breaking down fat and protein to provide substrates for liver glucose synthesis, and by inhibiting peripheral glucose uptake and utilization. In health, a perfect balance is maintained between the levels of insulin and its antagonists, so that glucose entry into the blood is precisely matched by the rates of glucose removal from the blood. In this way, a steady supply of glucose to the brain is assured. Hypoglycemia results when this balance is disturbed.

As blood glucose falls, a series of neurohumoral responses occur. In producing acute recovery from insulin-induced hypoglycemia, glucagon is probably the major hormone, but epinephrine alone can also produce near-normal recovery (5). Stimulation of the sympathetic nervous system, reflected indirectly by rising norepinephrine levels, is also important, especially in the

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IDDM, insulin-dependent diabetes mellitus; EEG, electroencephalogram.

Table 1—*Glucose thresholds for counterregulatory responses*

	Subjects		
	Nondiabetic	Diabetic	Diabetic on intensified therapy
Epinephrine	3.3–3.5	3.4–3.9	2.6
Subjective awareness	2.7–2.9	3.2–4.3	2.4
Cortical EEG	<2.0	≤2.0	2.0
Deterioration in:			
Psychomotor performance	2.6–2.9	2.6	—
P300 changes	2.6–4.0	2.5	—

Data from literature, including refs 20,43,51–53,55–57, and 60.

generation of warning symptoms of hypoglycemia. In more prolonged hypoglycemia, the actions of growth hormone and cortisol assume increasing importance, and clinical syndromes of hypoglycemia occur in their absence (6–8). These neurohumoral responses are important not just in producing recovery from insulin-induced hypoglycemia in the clinic or laboratory, but in defending against hypoglycemia during times of increased glucose utilization (e.g., during exercise) (9,10), and when the exogenous glucose supply is limited (e.g., during prolonged fast) (11,12), or in periods of postprandial hyperinsulinemia (13). The responses are more vigorous in children than in adults (14).

Because glucose counterregulation depends on adequate glycogenolysis and, if prolonged, gluconeogenesis, factors that interfere with these will interfere with the efficiency of glucose recovery from hypoglycemia. Likewise, intellectual appreciation of developing hypoglycemia and a coordinated appropriate feeding response also are necessary to defend against neuroglycopenia. One agent that will interfere with both of these processes is alcohol, which inhibits gluconeogenesis and impairs subjective awareness of otherwise apparently normal hormonal and neurological responses (15). In addition, β-blockade can interfere with counterregulation and alter symptom generation in hypoglycemia (16–19).

In general, during progressive hypoglycemia, adrenergic and symptomatic responses occur at higher glucose levels than those at which cortical dysfunction can be detected (Table 1), thus playing a protective role (20). There is, however, no direct correlation between adrenergic responses to hypoglycemia and symptomatic awareness. In prolonged but stable hypoglycemia, epinephrine levels remain high, but subjective awareness is diminished (21).

What triggers counterregulatory responses to hypoglycemia? Experimental evidence suggests that recognition of a falling blood glucose level occurs in the brain and that counterregulation is initiated from a cerebral center. A cerebral glucose sensor has been postulated. Its location is not known, but it is believed to be in the hypothalamus (22) or possibly the brain stem (23). In dogs, peripheral counterregulatory responses to hypoglycemia are almost abolished if cerebral glucose supplies are maintained during the induction of peripheral hypoglycemia (24).

The evidence suggests that counterregulation is triggered in response to a fall in the rate of cerebral metabolism, secondary to a failure of cerebral glucose supply. Thus, in animals (25,26) and humans (27), hypoglycemic responses can be diminished by provision of an alternate cerebral metabolic fuel, which, presumably, supports cerebral metabolism during glucose deprivation. The hyperketonemia of prolonged fasting reduces peripheral responses to hypoglycemia (28). The effect of such fuels on cognitive function is unknown.

Cerebral glucose uptake is by and large independent of insulin action. Whereas the rate of glucose uptake into and utilization by peripheral tissues (e.g., muscle and fat) are extremely insulin sensitive, brain glucose uptake is conventionally considered insulin independent. Yet insulin receptors are found in the brain. And, it is possible that high levels of insulin may encourage additional glucose movement into the brain. The evidence in humans is conflicting (29). At least one group has described diminished counterregulatory responses to hypoglycemia in humans (30), implying that, in the presence of very high insulin levels, insulin can help support intracerebral glucose supplies and metabolism during peripheral hypoglycemia.

IN IDDM— Counterregulatory responses to hypoglycemia are impaired in patients with diabetes. Glucose recovery from hypoglycemia is impaired by the persistence of the insulin effect and, in nondiabetic individuals, reduced pancreatic insulin secretion is an important part of the glucoregulatory response. In diabetes, circulating insulin levels depend entirely on the timing and nature of previous insulin administration and cannot respond to changes in ambient glucose levels. Persistence of circulating insulin also is enhanced by the presence of insulin-binding antibodies; these act as a reservoir for insulin, releasing it gradually, long after injection (31). Patients with insulin antibodies may be at increased risk of hypoglycemia, secondary to delayed insulin clearance (32,33). Many investigators have shown that patients with established diabetes lose their glucagon response specifically to hypoglycemia but not to other stimuli (32,34). Even children, whose responses to hypoglycemia are generally enhanced, show this loss of glucagon response to

hypoglycemia if they have had diabetes for a few years (14,35). This loss of one of the prime movers of glucose counterregulation enhances risk of hypoglycemia. Finally, a significant number of diabetic patients loses epinephrine and sympathetic responses to hypoglycemia. This might place them at special risk, not only because of failure of spontaneous glucose recovery, but because of the diminished warning symptoms of developing hypoglycemia (36,37).

Counterregulatory failure detected during experimentally induced hypoglycemia is associated with an increased risk of severe hypoglycemia in daily life (36–38); this risk increases with increasing duration of diabetes (38). Unexpectedly, no strong and absolute correlation is evident between autonomic failure and increased incidence of severe or asymptomatic hypoglycemia (38)—possibly because the diminished adrenergic response of such patients (39) is associated with an increased sensitivity to circulating catecholamines—an upregulation of receptors (40). Apart from increasing the duration of diabetes (41), other well-documented associations with severe hypoglycemia and relative failure of glucose counterregulation are previous severe hypoglycemia (37,38) and intensified insulin therapy (42). Intensified insulin therapy and/or tight metabolic control are persistently associated with increased risk of severe hypoglycemia (42). One possible explanation for this might be the delay in onset of adrenergic responses to hypoglycemia seen in such patients. In experimental hypoglycemia, catecholamine responses to and symptoms of hypoglycemia are initiated later (i.e., at a lower blood glucose level) in patients receiving intensified insulin therapy (in good metabolic control) than in more loosely controlled diabetic or nondiabetic subjects (43,44). Further, defective counterregulatory responses can be induced in unselected diabetic patients by instituting stricter metabolic control (43). Similar impairment of counterregulation has recently been induced in nondiabetic healthy volunteer subjects by the induction of hypoglycemia on the afternoon of the day before testing (45), suggesting that recurrent hypoglycemia in daily life may contribute to that defect. This acute induction of defects in counterregulatory responses has now been reproduced in subjects with diabetes by prior hypoglycemia (46). Of interest is that similarly impaired counterregulatory responses in 2 patients with recurrent hypoglycemia, resulting from insulinomas, have been restored by removal of the tumor and restoration of chronic normoglycemia (47,48). Very recently, some restoration of symptomatic responses to hypoglycemia has been demonstrated in diabetic patients with hypoglycemia unawareness by altering their diabetic treatment to avoid all hypoglycemia for a while (49).

Animals with experimentally induced chronic hypoglycemia show an increase in the number of cerebral glucose transporters and in the efficiency of cerebral glucose extraction from the blood (50). These animals maintain intracerebral glucose levels and cerebral metabolic rate more efficiently during subsequent hypoglycemia. It has been suggested that the impaired counterregulatory responses from intensified insulin therapy and strict diabetic control may be a result of a similar increase in efficiency of glucose extraction by the putative glucose sensor in the brain. If this is true, cognitive function should be better preserved at low blood glucose levels in subjects with this type of defective counterregulation. Unfortunately, delayed counterregulatory responses to hypoglycemia are not matched by a delayed onset of cerebral dysfunction attributable to neuroglycopenia (51–53). These factors intensify the vulnerability of these patients to severe hypoglycemia.

COGNITIVE FUNCTION AND DYSFUNCTION IN HYPOGLYCEMIA— The most dangerous consequence of hypoglycemia in insulin-treated diabetes is impaired intellectual or cognitive function. Severe hypoglycemia is defined as hypoglycemia in which treatment has to be administered by someone other than the patient, usually because disordered intellectual function makes it impossible for the victim to self-medicate. This can be extremely dangerous if it occurs at a time when concentration and good intellectual function is essential, for example, while driving. Some degree of neuroglycopenia is implicit in all hypoglycemic episodes, and mild abnormalities of brain function can be distinguished during very minor degrees of hypoglycemia (see below). However, it is the failure of higher cerebral function during hypoglycemia that causes the most concern.

Brain function is not an easy parameter to measure with any degree of objectivity or precision. Several methods have been used to evaluate cerebral function and the factors that might influence it during hypoglycemia.

Severe neuroglycopenia is associated with changes in the cortical EEG. Slowing of the EEG and the occurrence of delta-waves in the resting, awake EEG are seen in healthy and diabetic individuals as blood glucose falls below 2 mM (51,54,55). These changes are not specific for hypoglycemia, but, in the absence of drowsiness or other pathology, they can be used to indicate the onset of cortical neuroglycopenia. Occasionally, there are intensively treated diabetic patients who appear to tolerate low blood glucose levels more or less with impunity; it is tempting to speculate that their brains have in some way become more resistant to neuroglycopenia. This does not appear to be the case. Intensively treated patients with a lowered glucose threshold for counterregulatory or symptomatic responses to hypoglycemia actually develop EEG changes at the same, or even higher, blood glucose levels than poorly controlled patients or nondiabetic control subjects (51). Further, psychomotor performance and cognitive function deteriorate at similar blood glu-

cose levels in subjects with different previous glycemic histories, or different glucose levels that have previously been associated with adrenergic counterregulatory responses (52,53).

More subtle changes in neurophysiology can be seen by measuring cortical evoked potentials during hypoglycemia. These potentials are the cortical electrical responses to external stimuli processed by the brain. Increased latency and diminished magnitude of the P300, one component of the cortical evoked responses, have been noted by one group of observers at a blood glucose level of 4 mM (56). However, most investigators find changes in latency and amplitude of the P300 at glucose levels similar to or slightly higher than those associated with early adrenergic responses (57–60). Indeed, some have found a close association between P300 changes and adrenergic responses to hypoglycemia in patients with intensively treated diabetes and late onset of counterregulatory responses (61,62). These data suggest that there is global adaptation of brain function to hypoglycemia in such patients, which explains the delayed stimulation of counterregulatory responses and the delayed deterioration in P300. However, these data are in contrast with the finding that other measures of cognitive function (EEG, neurophysiological tests, 4-choice reaction time) deteriorate at similar glucose levels in all subjects (51–53), which suggests a lack of protective adaptation of at least some aspects of brain function in hypoglycemia-unaware patients.

The clinical significance and correlates of P300 responses remain obscure; however, their relatively early appearance during progressive hypoglycemia suggests that they could mark a relatively minor change in neuronal function associated with initiation of counterregulation. The clinical association between defective counterregulatory responses and increased risk of asymptomatic hypoglycemia and severe hypoglycemia suggests that any adaptation of higher brain function to recurrent hypoglycemia is not substantial.

Other more direct measures of cognitive function during acute hypoglycemia include measurement of reaction times (63,64) and performance scores on set tasks (64–66). At hypoglycemia, reaction times are slowed and performance on certain neuropsychological tests is impaired (63–66). Four-choice reaction time may be a particularly reproducible test. The use of such performance tests, as mentioned above, indicates a failure of adaptation of cortical function in hypoglycemia-prone patients with delayed onset of counterregulation (52,53), which would fit with the clinical experience of patients with subjective unawareness of hypoglycemia associated with increased risk of severe neuroglycopenia.

Neurohumoral responses to hypoglycemia, especially catecholamine and adrenergic responses, resolve rapidly as hypoglycemia remits. Recovery of cognitive function and psychomotor performance lags behind this, and impaired performance can be detected even after glucose recovery and restoration of hormonal status (67). However, full recovery can be demonstrated on subsequent testing (67).

Much of current research centers on measurement of brain function during acute hypoglycemia with the aim of either elucidating its pathophysiology or of developing a hypoglycemia alarm. It was hoped that neurophysiological monitoring might provide the basis for such a hypoglycemia warning system, but this hope has yet to be realized. The possibility of training patients to better recognize subtle and different clues for developing hypoglycemia, including the use of simple cognitive function tests, may, however, be realized (68).

There has been less research into the effect of hypoglycemia on long-term cognitive function. This has proved extremely difficult to assess, partly because of the difficulty in evaluating the premorbid state, the effects of diabetes per se (69), and the difficulties in determining the cause and effect in patients with either recurrent severe hypoglycemia or intellectual dysfunction. Some evidence, however, suggests that recurrent severe hypoglycemia in diabetes may be associated with permanent neurological sequelae (70) and that cognitive impairment possibly related to chronic microvascular complications of diabetes may be exacerbated by severe hypoglycemia (71). Therefore, avoiding recurrent severe hypoglycemia becomes crucial.

HUMAN INSULIN, COUNTERREGULATION, AND COGNITIVE FUNCTION IN HYPOGLYCEMIA

— The previously rather academic arena of debate concerning cognitive responses to hypoglycemia has, since 1987, become an issue of public concern and media exposure. In that year, a Swiss group reported that loss of awareness of hypoglycemia was common in a group of long-standing patients converted to human insulin (72,73). These reports had a number of weaknesses (74). A more recent paper from the same group, which reported increased use of human insulin in patients admitted to the hospital with hypoglycemia (75), can also be criticized because metabolic control was much tighter in patients admitted with hypoglycemia than in control patients. In the same journal, however, the authors also reported a study in which loss of concentration moved from fourth to first place in order of symptom importance to the patient during hypoglycemia (76). Nevertheless, hypoglycemic unawareness could not be correlated with use of human insulin in studies in Edinburgh (77), Germany (78), Denmark (79), Pittsburgh (80), or the U.S. in general (81). These studies were retrospective, but a recent prospective clinical study in Australia also has been negative (82). The problem of loss of awareness of hypoglycemia, and any pos-

sible relationship to the use of human insulin, is discussed elsewhere in this supplement but it may be instructive to examine research that has been done using different species of insulin to induce hypoglycemia. The evidence is somewhat conflicting (83–86), although most recent reports find no difference between responses to experimentally induced hypoglycemia produced by human or porcine insulin (87–94). Occasional patients do seem to fare better on pork insulin, even when unable to distinguish between the species under conditions of controlled hypoglycemia (93). One group experienced fewer symptoms at blood glucose levels of 3.3 mM when inducing hypoglycemia with human insulins, although at 2.6 mM there were no differences, and other groups found no differences at all (93,94). A recent report suggested that hypoglycemia induced by human insulin had transiently less effect on P300 than that induced by pork insulin (59). This finding, which has yet to be confirmed, might be explained on the grounds that human insulin is more lipophilic than pork and, therefore, would penetrate nerve tissue more rapidly, although it remains unclear why this should diminish warning symptoms. The evidence of an effect of the insulin species itself as the direct cause of the problem is lacking, although with the inability of science to prove a negative association, patients should be allowed to choose their insulin species.

Perhaps the most important moral to be drawn at this stage of the controversy is that cognitive function in hypoglycemia, despite the complexity of the issues involved, is a matter of urgent concern to many patients with diabetes. The ability to recognize hypoglycemia is for them the difference between safety and danger while driving, peace of mind and anxiety when they lie alone at night, and an independent life or an embarrassing reliance on others. The need to deliver good glycemic control without threatening this precious lifeline constitutes the major challenge of modern insulin therapy.

References

1. Sokoloff L: Metabolism of the central nervous system in vivo. In *Handbook of Physiology*. Section Neurophysiology. Baltimore, Waverly Press, 1960, p.1843
2. Crone C: Facilitated transfer of glucose from blood into brain tissue. *J Physiol* 181:103–13, 1965
3. Garber AJ, Cryer PE, Santiago JV, Haymond MW, Pagliara AS, Kipnis DM: The role of adrenergic mechanisms in the substrate and hormonal response to insulin-induced hypoglycemia in normal man. *J Clin Invest* 58:7–15, 1976
4. Gerich J, Cryer PE, Rizza R: Hormonal mechanisms in acute glucose counterregulation: the relative roles of glucagon, epinephrine, norepinephrine, growth hormone and cortisol. *Metabolism* 29: 1164–75, 1980
5. Rizza RA, Cryer PE, Gerich JE: Role of glucagon, catecholamines and growth hormone in human glucose counterregulation. *J Clin Invest* 64:62–71, 1979
6. DeFeo P, Perriello G, Torlone E, Ventura MM, Sarteusario F, Brunetti P, Gerich JE, Bolli GB: Demonstration of a role for growth hormone in glucose counterregulation. *Am J Physiol* 256: E835–43, 1989
7. Boyle PJ, Cryer PE: Roles of growth hormone and cortisol in defence against prolonged hypoglycemia. *Diabetes* 39 (Suppl. 1):5A, 1990
8. Haymond MW, Karl I, Weldon VV, Ragliara AS: The role of growth hormone and cortisone on glucose and gluconeogenic substrate regulation in fasted hypopituitary children. *J Clin Endocrinol Metab* 42:846–56, 1976
9. Hirsch IB, Marker JC, Smith LJ, Spina RJ, Parvin CA, Holloszy JO, Cryer PE: Roles of insulin and glucose in the prevention of hypoglycemia during exercise in humans. *Am J Physiol* 260: E695–704, 1991
10. Simonson DC, Koivisto V, Sherwin RS, et al: Adrenergic blockade alters glucose kinetics during exercise in insulin dependent diabetics. *J Clin Invest* 73:1648–58, 1984
11. Merimee TJ, Tyson JE: Stabilization of plasma glucose during fasting. Normal variation in two separate studies. *N Engl J Med* 291:1275–78, 1974
12. Bolli G, Gottesman I, Cryer P, Gerich J: Glucose counterregulation during prolonged hypoglycemia in normal humans. *Am J Physiol* 247:E206–14, 1984
13. Tse TE, Clutter WE, Shah SD, Cryer PE: Mechanisms of postprandial glucose counterregulation in man. *J Clin Invest* 72:278–86, 1983
14. Amiel SA, Simonson DC, Sherwin RS, Lauritano AA, Tamborlane WV: Exaggerated epinephrine responses to hypoglycemia in children and adolescents. *J Pediatr* 110:832–37, 1987
15. Kerr D, Macdonald IA, Heller SR, Tattersall RB: Alcohol causes hypoglycemic unawareness in healthy volunteers and patients with type I (insulin-dependent) diabetes. *Diabetologia* 33:216–21, 1990
16. Frier BM, Corrall RJM, O'Brien IAD, Lewin IG, Hay ID, Roland J: Hypoglycemia during adrenergic beta-blockade: evidence against mediation via a deficiency of lactate for gluconeogenesis. *Metabolism* 34:1039–43, 1985
17. Popp DA, Tse TF, Shah S, Clutter WE, Cryer PE: Oral propranolol and metoprolol both impair glucose recovery from insulin induced hypoglycemia in insulin dependent diabetes mellitus. *Diabetes Care* 7:243–47, 1984
18. Macdonald IA, Bennett T, Gale EAM, Walford S: The effect of propranolol or metoprolol on thermoregulation during insulin induced hypoglycemia in man. *Clin Sci* 63:301–30, 1982
19. Molner GW, Read RC: Propranolol enhancement of hypoglycemic sweating. *Clin Pharmacol Ther* 15:409–96, 1974
20. Boyle PJ, Schwartz NS, Shah SD, Clutter WE, Cryer PE: Plasma glucose concentrations at the onset of hypoglycemic symptoms in patients with poorly controlled diabetes and in non-diabetics. *N Engl J Med* 318:1487–92, 1988
21. Kerr D, Macdonald IA, Tattersall RB: Adaptation to mild hypoglycemia in normal subjects despite sustained increases in counterregulatory hormones. *Diabetologia* 32:249–54, 1989
22. Benzo CA: The hypothalamus and blood

glucose regulation. *Life Sci* 32:2509–15, 1983
23. Ritter RC, Slusser PG, Stone S: Glucoreceptors controlling feedback and blood glucose: location in the hindbrain. *Science* 213:451–53, 1981
24. Biggers DW, Myers SR, Neal D, Stinson R, Cooper N, Jaspan JB, Williams PE, Cherrington AD, Frizzell RT: Role of the brain in counterregulation of insulin induced hypoglycemia in dogs. *Diabetes* 38:7–16, 1989
25. Flatt JP, Blackburn GL, Randers G, Stanbury JB: Effects of ketone body infusion on hypoglycemic reaction in post absorptive dogs. *Metabolism* 23:151–58, 1974
26. Avogaro A, Nosadini R, Doria A, et al: Substrate availability other than glucose in the brain during euglycemia and insulin induced hypoglycemia in dogs. *Metabolism* 39:46–50, 1990
27. Amiel SA, Archibald HR, Chusney G, Gale EAM: Ketones diminish hormone responses to hypoglycemia—evidence for acute adaptation of cerebral metabolism to a non-glucose fuel? *Diabetologia* 32:460A, 1989
28. Drenick EJ, Alvarez LC, Tamasi GC, Brickman AS: Resistance to symptomatic insulin reactions after prolonged fasting. *J Clin Invest* 51:2754–62, 1972
29. Diamond MP, Hallarman L, Zych K, Jones T, Howard M, Tamborlane WV, Sherwin RS: Insulin per se suppresses counterregulatory hormone responses to hypoglycemia. *Diabetes* 39 (Suppl. 1):4A, 1990
30. Davis SN, Dobbins R, Nadaud S, Neal D, Williams P, Cherrington A: The effects of direct insulin sensing by the brain during hypoglycemia. *Diabetes* 40 (Suppl. 1): 154A, 1991
31. van Haeften TW, Bolli GB, Dimitradis GP, Gottesman I, Horwitz D, Gerich JE: Effect of insulin antibodies and their kinetic characteristics on plasma free insulin dynamics in diabetic patients with insulin antibodies. *Metabolism* 35:649–56, 1986
32. Bolli G, DeFeo P, Compagnucci P, Cartechimi MG, Angeletti G, Santeusanio F, Brumetti P, Gerich JE: Abnormal glucose counterregulation in insulin-dependent diabetes mellitus: interaction of anti-insulin antibodies and impaired glucagon secretion. *Diabetes* 32:134–41, 1983
33. Wilkin T, Keller U, Diaz JL, Armitage M: Delayed disappearance of human porcine insulin in a patient with the insulin autoimmune syndrome. *Diabetes Res Clin Pract* 8:131–36, 1990
34. Gerich JE, Langloisd M, Noacco C, Karam JH, Forsham PH: Lack of a glucagon response to hypoglycemia in diabetes: evidence for an intrinsic pancreatic alpha-cell defect. *Science* 182:171–73, 1973
35. Singer-Granick C, Hoffman RP, Kerensky K, Drash AL, Becker D: Glucagon responses to hypoglycemia in children and adolescents with IDDM. *Diabetes Care* 11:643–49, 1988
36. Clarke WL, Gonder-Frederick LA, Richards FE, Cryer PE: Multifactorial origin of hypoglycemic symptom unawares in IDDM: association with defective glucose counterregulation and better glycemic control. *Diabetes* 40:680–85, 1991
37. Sjorborn NC, Adamson V, Lins PE: The prevalence of impaired glucose counterregulation during an insulin infusion test in insulin treated diabetic patients prone to severe hypoglycemia. *Diabetologia* 32: 818–25, 1989
38. Ryder RJ, Owens DR, Hayes TM, Chatei MA, Bloom SR: Unawareness of hypoglycemia and inadequate hypoglycemic counterregulation: no causal relationship with diabetic autonomic neuropathy. *Br Med J* 301:783–87, 1990
39. Hoeldtke RD, Boden G, Shuman CR, Owen OE: Reduced epinephrine secretion and hypoglycemia unawareness in diabetic autonomic neuropathy. *Ann Intern Med* 73:403–11, 1982
40. Hilsted J, Richter E, Madsbad S, Tronier B, Christensen NJ, Hildebrandt P, Damkjaer M, Galbo H: Metabolic and cardiovascular responses to epinephrine in diabetic autonomic neuropathy. *N Engl J Med* 317:421–26, 1987
41. Hepburn DA, Patrick AW, Eadington DW, Ewing DJ, Frier BM: Unawareness of hypoglycemia in insulin-treated diabetic patients: prevalence and relationship to autonomic neuropathy. *Diabetic Med* 7:711–17, 1990
42. The DCCT Research Group: Diabetes Control and Complications Trial (DCCT): results of the feasibility study. *Diabetes Care* 10:1–10, 1987
43. Amiel SA, Tamborlane WV, Simonson DC, Sherwin RS: Effect of intensified insulin therapy on glycemic thresholds for counterregulatory hormone release. *Diabetes* 37:901–907, 1988
44. Amiel SA, Simonson DC, Tamborlane WV, Sherwin RS: Defective glucose counterregulation after strict glycemic control of insulin-dependent diabetes mellitus. *New Engl J Med* 316:1376–83, 1987
45. Heller SR, Cryer PE: Reduced neuroendocrine and symptomatic responses to subsequent hypoglycemia in non-diabetic humans. *Diabetes* 40:223–26, 1991
46. Widom B, Simonson DC: Intermittent hypoglycemia impairs glucose counterregulation. *Diabetes* 41:1597–602, 1992
47. Davis M, Shamoon H: Deficient counterregulatory hormone responses during hypoglycemia in a patient with insulinoma. *J Clin Endocrinol Metab* 72:788–92, 1991
48. Maran A, Macdonald IA, Taylor J, Amiel SA: Reversibility of asymptomatic hypoglycaemia in man: a case report. *Diabetologia* 34 (Suppl. 2):A189, 1991
49. Fanelli C, Rambotti AM, Epifano L, Calderone S, Porcellati F, DeFeo P, Bolli G: Short-term reversibility of hypoglycemia unawareness in IDDM. *Diabetes* 41 (Suppl. 1):119, 1992
50. McCall AL, Fixman LB, Fleming N, Tornheim K, Chick W, Ruderman N: Chronic hypoglycemia increases brain glucose transport. *Am J Physiol* 251: E442–47, 1986
51. Amiel SA, Pottinger R, Cunnah DC, Archibald HR, Chusney G, Prior P, Gale EAM: Effect of antecedent glucose control on cerebral function during hypoglycemia. *Diabetes Care* 14:109–18, 1991
52. Widom B, Simonson DC: Glycemic control and neurophysiologic function during hypoglycemia in patients with insulin dependent diabetes mellitus. *Ann Intern Med* 112: 904–12, 1990
53. Maran A, Lomas J, Macdonald IA, Amiel

SA: Lack of protection of cerebral function in well controlled diabetic patients with hypoglycemia (Abstract). 42 (Suppl.1): *Diabetes* 17A, 1993. In press
54. Himwich HE, Hadidian Z, Fazekas JF, Hoagland H: Cerebral metabolism and electrical activity during insulin hypoglycemia in man. *Am J Physiol* 125:578–85, 1939
55. Pramming S, Thorsteinsson B, Stigsby B, Binder C: Glycemic threshold for changes in electroencephalograms during hypoglycemia in patients with insulin dependent diabetes mellitus. *Br Med J* 296:665–67, 1988
56. DeFeo P, Gallai V, Mazzotta G, et al: Modest decrements in plasma glucose concentration causes early impairment of cognitive function and later activation of glucose counterregulation in the absence of hypoglycemic symptoms in normal man. *J Clin Invest* 82:436–44, 1988
57. Tallroth G, Lindgren M, Stenberg G, Rosen I, Agardh C-D: Neurophysiological changes during insulin induced hypoglycemia and in the recovery period following glucose infusion in Type 1 diabetes mellitus and normal man. *Diabetologia* 33:319–23, 1990
58. Blackman JD, Towle VL, Lewis GF, Spire JF, Polonsky KS: Hypoglycemic thresholds for cognitive function in humans. *Diabetes* 39:828–35, 1990
59. Kern W, Lieb K, Kerner W, Born J, Fehm HL: Differential effects of human and pork insulin induced hypoglycemia in neuronal functions in humans. *Diabetes* 39:1091–98, 1990
60. Blackman JD, Towle VL, Sturis J, Lewis GF, Spire JP, Polonsky KS: Hypoglycemic thresholds for cognitive dysfunction in IDDM. *Diabetes* 41:392–99, 1992
61. Jones TW, McCarthy G, Tamborlane WV, Caprio S, Roessler E, Kraemer D, Starick-Zych K, Allison T, Boulware SD, Sherwin RS: Mild hypoglycemia and impairment of brain stem and cortical evoked potentials in healthy subjects. *Diabetes* 39:1550–55, 1990
62. Ziegler D, Hubinger A, Muhlen H, Gries FA: Effect of previous glycaemic control on the onset and the magnitude of cognitive dysfunction during hypoglycaemia in type one (insulin dependent) diabetic patients. *Diabetologia* 35:828–34, 1992
63. Herold KC, Polonsky KS, Cohen RM, Levy J, Douglas F: Variable deterioration in cortical function during insulin-induced hypoglycemia. *Diabetes* 34:677–85, 1985
64. Holmes CS, Hayford JT, Gonzalez JL, Weydert JA: A survey of cognitive functioning at different glucose levels in diabetic patients. *Diabetes Care* 6:180–85, 1983
65. Holmes CS, Keopke KM, Thompson RG, Gyves PW, Weydert JA: Verbal fluency and naming performance in type i diabetes at different blood glucose concentrations. *Diabetes Care* 7:454–59, 1984
66. Pramming S, Thorsteinsson B, Theilgaard A, Pinner EM, Binder C: Cognitive function during hypoglycemia in type 1 diabetes mellitus. *Br Med J* 292:647–50, 1986
67. Puczynski MS, Puczynski SS, Reich J, Kaspar JC, Emanuele MA: Mental efficiency and hypoglycemia. *J Dev Behav Pediatr* 11:170–74, 1990
68. Cox DJ, Gonder-Frederick L, Autoun B, Cryer PE, Clarke WE: Perceived symptoms in the recognition of hypoglycemia. *Diabetes Care* 16:519–27, 1993
69. Prescott JH, Richardson JT, Gillespie CR: Cognitive function in diabetes mellitus: the effects of duration of illness and glycemic control. *Br J Clin Psychol* 29 (Pt. 2):167–75, 1990
70. Wredling R, Levander S, Adamson U, Lins PE: Permanent neurophysiological impairment after recurrent episodes of severe hypoglycemia in man. *Diabetologia* 33:152–57, 1990
71. Ryan CM, Williams TM, Finegold DN, Orchard TJ: Cognitive dysfunction in adults with type I (insulin-dependent) diabetes mellitus of long duration: effects of recurrent hypoglycaemia and other chronic complications. *Diabetologia* 36: 329–34, 1993
72. Berger WG, Althaus BV: Reduced awareness of hypoglycemia after changing from porcine to human insulin in IDDM. *Diabetes Care* 10:260–61, 1987
73. Teuscher A, Berger WG: Hypoglycemia unawareness in diabetics transferred from beef/porcine to human insulin. *Lancet* ii:382–85, 1987
74. Gale EAM: Hypoglycaemia and human insulin. *Lancet* ii:1264–66, 1989
75. Egger M, Davey Smith G, Imhoof H, Teuscher A: Risk of severe hypoglycemia in insulin treated diabetic patients transferred to human insulin: a case control study. *Br Med J* 303:617–21, 1991
76. Egger M, Smith GD, Teuscher AU, Teuscher A: Influence of human insulin on symptoms and awareness of hypoglycaemia: a randomised double blind crossover trial. *Br Med J* 303:622–26, 1991
77. Hepburn DA, Eadington DW, Patrick AW, Colledge NR, Frier BM: Symptomatic awareness of hypoglycemia: does it change in transfer to human insulin? *Diabetic Med* 6:586–90, 1989
78. Mulhauser I, Heinemann L, Fritsche E, von Lennep K, Berger M: Hypoglycemic symptoms and frequency of severe hypoglycemia in patients treated with human and animal insulin preparations. *Diabetes Care* 14:745–50, 1991
79. Dejgaard A: Unawareness to hypoglycemia with human insulin—facts and fictions experiences with Scandinavia and other European countries. In *Hypoglycemia and Human Insulin*. Federlin K, Keen H, Mehnert H, Eds. Stuttgart, Georg Thieme Verlag, 1990, p. 135–39
80. Orchard TJ, Maser RE, Becker DJ, Dorman JS, Drash AL: Human insulin use and hypoglycemia: insights from the Pittsburgh Epidemiology of Diabetes Complications Study. *Diabetic Med* 9:469–74, 1991
81. The DCCT Research Group: Epidemiology of severe hypoglycemia in the diabetes control and complications trial. *Am J Med* 90:450–59, 1991
82. Colagiuri S, Miller J, Petrocz P: Double-blind crossover comparison of human and porcine insulins in patients reporting lack of hypoglycaemia awareness. *Lancet* 339:1432–35, 1992
83. Heine RJ, Van der Heyden EAP, Van der Veen EA: Responses to human and porcine insulin in healthy subjects. *Lancet* ii:946–48, 1989
84. Schluter KJ, Petersen KG, Sontheimer J, Enzmann F, Kerp L: Differential counterregulatory responses to human insulin (recombinant DNA) and purified pork

insulin. *Diabetes Care* 5 (Suppl. 2):78–81, 1982
85. Rosak C, Althoff PH, Enzmann F, Schoffling K: Comparative studies on intermediary metabolism and hormonal counterregulation following human insulin (recombinant DNA) and purified pork insulin in man. *Diabetes Care* 5 (Suppl. 2):82–89, 1982
86. Petersen KG, Schluter KJ, Kerp L: Less pronounced changes in serum potassium and epinephrine during hypoglycemia induced by human insulin (recombinant DNA). *Diabetes Care* 5:90–92, 1982
87. Owens DR, Vora JP, Tronier B, Keller U, Luzio S, Turkes A: Hormonal counterregulatory responses to human (semisynthetic and recombinant DNA) and porcine insulin induced hypoglycemia. *Diabetes Res* 8:17–20, 1988
88. Perez Fernandez R, Casaneuva FF, Devesa J, Cabezas Cerrato J: Metabolic and hormonal parameters after insulin induced hypoglycemia in man, comparison between biosynthetic human insulin and purified pork insulin. *Horm Metab Res* 17:351–54, 1985
89. Bendson I, Binder C: Counterregulatory hormone responses to insulin-induced hypoglycemia in IDDM patients. A comparison of equimolar amounts of porcine and semisynthetic human insulin. *J Intern Med* 229:293–96, 1991
90. Clausen Sjorborn N, Lins PE, Adamson V, Theodorsson E: A comparative study of the hormonal responses to insulin-induced hypoglycemia using semi-synthetic and pork insulin in patients with insulin dependent diabetes mellitus. *Diabetic Med* 7:775–79, 1990
91. Jones TW, Caprio S, Diamond MP, Hallarman L, Boulware S, Sherwin RS, Tamborlane WV: Does insulin species modify counterregulatory hormone responses to hypoglycemia? *Diabetes Care* 14:728–31, 1991
92. Patrick AW, Bodmer CW, Tieszen KL, White MC, Williams G: Human insulin and awareness of acute hypoglycemia symptoms in insulin dependent diabetes. *Lancet* 338:528–32, 1991
93. Maran A, Lomas J, Archibald H, Macdonald IA, Gale EAM, Amiel SA: Double blind clinical and laboratory study of hypoglycaemia with human and porcine insulin in diabetic patients reporting hypoglycaemia unawareness after transferring to human insulin. *Br Med J* 306:167–71, 1993
94. Lingenfelser T, Buettner UW, Plonz C, Steffen J, Eggstein M, Jakober B: Hormonal counterregulation, symptom awareness, and neurophysiological function in type 1 diabetes during insulin-induced hypoglycaemia. *Diabetic Med* 9:528–35, 1992