Hypoglycemia and the Central Nervous System

a report by

Erwin C Puente,[1] **Tariq Tanoli**, **MD**[1] *and* **Simon J Fisher**, **MD**, **PhD**[1,2]

1. Division of Endocrinology, Metabolism, and Lipid Research, Department of Medicine, Washington University in St Louis;
2. Department of Cell Biology and Physiology, Washington University in St Louis

DOI: 10.17925/USE.2008.04.2.38

The Diabetes Control and Complications Trial (DCCT) convincingly demonstrated that intensive insulin therapy reduces the microvascular complications associated with diabetes, including retinopathy, neuropathy, and nephropathy.[1,2] However, intensive insulin therapy that lowers blood glucose towards normal levels also significantly increases the risk for hypoglycemia. Moderate hypoglycemia can acutely affect cognitive ability, leading to temporary stupor and confusion. Severe hypoglycemia can lead to seizures, coma, and even death. Therefore, hypoglycemia causes recurrent morbidity that can interfere with the ability of individuals to work, as well as to perform activities of daily living.[3] The fear of a hypoglycemic reaction is also a barrier to intensive insulin therapy.

Hypoglycemia is not limited to patients with type 1 diabetes, but frequently occurs in patients with type 2 diabetes as well.[4,5] As the rate-limiting step for insulin therapy in diabetes, hypoglycemia precludes the maintenance of long-term euglycemia and the long-term benefits associated with tight glucose control. Recognizing the impact of hypoglycemia and the development of novel therapies aimed at reducing iatrogenic hypoglycemia are all vital to improve blood sugar management and improve the lives of people with diabetes.

Erwin C Puente is a senior MD/PhD student at Washington University in Saint Louis. He is registered in the Medical Scientist Training Program in the Division of Biology and Biomedical Sciences. He is performing his thesis work in molecular cellular biology, and his dissertation thesis involves research on insulin action and hypoglycemia.

Tariq Tanoli, MD, is a Clinical Fellow in the Division of Endocrinology, Metabolism, and Lipid Research at Washington University in St Louis. He is board-certified in internal medicine. Dr Tanoli graduated from Dow Medical University in Karachi, Pakistan and completed an internal medicine residency at St Luke's Hospital, Chesterfield.

Simon J Fisher, MD, PhD, is an Assistant Professor of Medicine, Cell Biology, and Physiology in the Division of Endocrinology, Metabolism, and Lipid Research at Washington University in St Louis. He is board-certified in internal medicine, endocrinology, and metabolism, and is a member of the American Diabetes Association (ADA). Dr Fisher's laboratory focuses its research on insulin action.

E: sfisher@dom.wustl.edu

Physiological Defenses Against Hypoglycemia

Glucose is the major energy source for maintenance of brain metabolism and function; however, the brain has limited glucose reserves and needs a continuous supply of the monosaccharide.[6] Therefore, the body has developed multiple systems to maintain glucose delivery to the brain and prevent hypoglycemia (see *Table 1*). First, a hierarchical hormonal response exists in response to decreasing blood glucose levels.[7–9] As blood glucose drops below 80mg/dl, pancreatic β-cell insulin secretion is reduced. If blood glucose drops further, the pancreatic α-cell will secrete glucagon and the adrenal medulla will release epinephrine.[7–9] Both glucagon and epinephrine act rapidly to increase glucose availability and therefore are the two major counter-regulatory hormones. Cortisol and growth hormone are also released, but they are unable to prevent prolonged hypoglycemia if glucagon and epinephrine responses are absent.[10] In sensing hypoglycemia, the nutritionally deprived brain also stimulates the sympathetic nervous system, leading to neurogenic symptoms such as sweating, palpitations, tremulousness, anxiety, and hunger.[11] These symptoms prompt individuals to ingest food to increase blood sugar levels. If these defenses are unable to restore blood sugar levels, inadequate glucose supply to the brain leads to neuroglycopenic symptoms such as confusion, difficulty speaking, ataxia, paresthesias, headaches, seizures, and coma.[12]

Unfortunately, these defense mechanisms are often absent or impaired in individuals with type 1 or advanced type 2 diabetes[13] (see *Table 1*). The glucagon response to hypoglycemia is eventually lost in patients with type 1 diabetes[14–16] and can be impaired in individuals with long-standing type 2 diabetes.[17] Therefore, patients with diabetes must rely on epinephrine release from the adrenal medulla to correct hypoglycemia. However, this response may also be attenuated in both type 1 and 2 diabetes.[11,13,14] Furthermore, patients with diabetes are likely to have impaired awareness of their hypoglycemia, termed hypoglycemia unawareness (HU).[11,13,14] HU is the reduction of neurogenic symptoms at a given level of hypoglycemia. If patients are unable to recognize that they are hypoglycemic, they will not take measures to increase their blood sugar levels, such as ingesting carbohydrates. Thus, patients with diabetes are at increased risk for experiencing severe hypoglycemic events.

Several studies determined that the strongest risk factor for future hypoglycemia is the number of prior hypoglycemic events;[18] that is, individuals who have experienced antecedent hypoglycemia are at greater risk for future hypoglycemia. The reason for this, as several studies have demonstrated, is that a prior episode of hypoglycemia impairs the body's response to subsequent hypoglycemia (see *Table 2*). Antecedent hypoglycemia causes hypoglycemia-associated autonomic

© TOUCH BRIEFINGS 2008

failure (HAAF).[11,13,14,18] HAAF results from reduced sympathoadrenal responses, leading to, first, defective glucose counter- regulation (e.g. attenuated epinephrine response) and, second, HU.[11,13,14,18] Attenuated epinephrine responses increase the risk for severe hypoglycemia by 25-fold,[19] and patients with HU have a six-fold increased risk for developing severe hypoglycemia.[20]

This situation seems paradoxical in that the body adapts to hypoglycemia by limiting its ability to defend against subsequent hypoglycemia. Several studies have indicated that a central nervous system (CNS) adaptation occurs after an episode of hypoglycemia.[13,21] The brain adapts to episodic periods of lower levels of blood sugar and thus the glycemic threshold for initiating glucose counter-regulation and neurogenic symptoms is shifted to lower glucose levels. Thus, more profound levels of hypoglycemia must be reached before these counter-regulatory responses (CRRs) are elicited.

Mechanisms of Hypoglycemia-associated Autonomic Failure

The exact mechanisms for the shift in the glycemic threshold are not fully understood, but clinical and basic science research has begun to discover critical adaptations within the CNS that contribute to HAAF. Several possibilities have been investigated, including increased brain glucose transport,[22–26] enhanced glucose metabolism,[27,28] glycogen super-compensation,[29,30] and altered neuronal activity.[31–35] Although some studies have shown that glucose transport to the brain may be increased after prolonged hypoglycemia (greater than two days),[26] studies investigating individuals with HAAF and patients with type 1 diabetes found no change in global blood–brain glucose transport after antecedent hypoglycemia.[25] However, this does not exclude the possibility of altered glucose transport in specific areas of the brain that sense and respond to changing blood glucose levels such as the hypothalamus.

Enhanced glucose metabolism is another potential mechanism. With more efficient glucose metabolism, the brain could maintain energy levels despite lower blood-glucose concentrations. Increased glucokinase activity—glucokinase is the enzyme responsible for the first step in the metabolism of glucose—has been hypothesized to play a major role.[27,28] Interestingly, an infusion of fructose, which inhibits glucokinase activity, in patients without diabetes and patients with type 1 diabetes enhanced the CRR to hypoglycemia.[36,37] Apart from glucose, increased transport and metabolism of other fuels, such as monocarboxylic acids (i.e. lactate, pyruvate, acetate), may contribute to HAAF. Recent studies have indicated that neurons can indeed utilize other substrates apart from glucose.[38–40] As an alternative fuel source, it has been shown that acetate transport and metabolism was increased in individuals with well-controlled type 1 diabetes compared with patients without diabetes.[41] These alternative fuels may provide sufficient energy to the brain during hypoglycemia and, consequently, delay the initiation of CRRs.

Additionally, several studies have demonstrated the importance of central insulin signaling on the CRR to hypoglycemia. In animal studies, increased insulin concentrations in the brain amplified the CRR[42–44] and the absence of CNS insulin signaling resulted in impaired CRR.[45]

Increased glycogen content following hypoglycemia (termed glycogen supercompensation) may also contribute to HAAF.[13,29] Both animal and human studies have shown glycogen levels to increase above basal levels following hypoglycemia.[29,46] The increased brain glycogen may provide nutrients to the brain during subsequent hypoglycemia, thereby delaying initiation of the CRR and neurogenic symptoms. Antecedent hypoglycemia can also affect neuronal activity. Alterations on potassium channel (K_{ATP}) activity and on the inhibitory neurotransmitter gamma-aminobutyric acid (GABA) have been implicated in the pathogenesis of HAAF.[31–35] Taken together, many mechanisms may contribute to HAAF. Pharmacological agents that target the above-mentioned pathways will need to be assessed to determine which will be most useful in preventing hypoglycemia.

A novel potential therapy to enhance the CRR is the use of fluoxetine, a selective serotonin re-uptake inhibitor (SSRI) commonly used as an antidepressant.[47,48] Briscoe et al. demonstrated that administration of fluoxetine for six weeks in patients with type 1 diabetes significantly increases sympathetic outflow and epinephrine responses to hypoglycemia, although neurogenic symptoms to hypoglycemia were not altered.[47] As epinephrine is the main counter-regulatory hormone in patients with long-standing type 1 diabetes, fluoxetine is an attractive pharmacological agent

Table 1: Physiological Defenses Against Hypoglycemia and Impairments in Diabetes

Glycemia (mg/dl)	Physiological Response	Response in Diabetes
~80	Reduced insulin secretion	Absent
~70	Increased glucagon	Absent
~70	Increased epinephrine	Reduced (HAAF)
~60	Neurogenic symptoms (sweating, palpitations, hunger)	Reduced (hypoglycemia unawareness)

HAAF = hypoglycemia-associated autonomic failure.
A hierarchial hormonal response occurs in response to hypoglycemia.[7–9] First, insulin secretion from the pancreatic β-cell is reduced. Further drops in glucose lead to increased glucagon and epinephrine release.[7–9] Hypoglycemia can also activate the autonomic nervous system, leading to sweating, palpitations, and hunger.[7–9] These neurogenic symptoms stimulate food intake. Together, these responses act in concert to restore blood sugar levels. Unfortunately, in individuals with type 1 diabetes or with advanced type 2 diabetes, these protective mechanisms are often absent or reduced. These impaired defenses significantly increase the frequency and severity of hypoglycemia in patients with diabetes.

Table 2: Acute and Long-term Effects of Moderate and Severe Hypoglycemia

Depth of Hypoglycemia	Acute Effects	Long-term Consequences
Moderate hypoglycemia	Neurogenic and neuroglycopenic symptoms	Hypoglycemia-associated autonomic failure: • reduced epinephrine response • hypoglycemic unawareness Increased risk for future hypoglycemia
Severe hypoglycemia	Seizures, coma, or death	Adolescent and adults: no long-term cognitive impairment Young children: possible deficits in cognitive ability

Moderate hypoglycemia acutely leads to neurogenic symptoms (sweating, palpitations, hunger, etc.) as well as neuroglycopenic manifestations (confusion, headache, ataxia, etc.).[7–9] However, a bout of hypoglycemia impairs the body's defense against subsequent hypoglycemia, a syndrome termed hypoglycemia-associated autonomic failure (HAAF).[11,13,14] HAAF increases the risk for future hypoglycemia, especially severe hypoglycemia. Severe hypoglycemia is very dangerous as it can cause seizures, coma, or death. Several studies have investigated whether severe hypoglycemia has long-term consequences on cognition. In adolescents and adults, severe hypoglycemia does not appear to have any detrimental effects on long-term cognitive ability.[60,61] Whether severe hypoglycemia affects cognition in young children has yet to be resolved, although some studies suggest it can affect memory recall and verbal abilities.[66,67]

Table 3: Hypoglycemia Prevention—Physician and Patient Points for Discussion

Consistent monitoring	Check blood sugar before all meals, at bedtime, and before driving
	Record data: blood sugar, carbohydrates, insulin dosage, special events/considerations
	Review data: assess for patterns of hypoglycemia (time of day, association with types of meals/activities/exercise, weekdays versus weekends, post-menstrual)
	If night-time hypoglycemia is suspected, wake patient at 3am for a few nights to check blood glucose
	Consideration of a continuous glucose monitoring system with hypoglycemia alarms
Meals	Adjust insulin-to-carbohydrate ratio to avoid post-meal hypoglycemia
	Proper assessment of portion size and carbohydrate content
	Ideal insulin-to-carbohydrate ratio should reach target blood sugar three to four hours after meals
	Pre-meal glycemia may influence the timing delay between pre-meal insulin dosage and initiating of meal
	If pre-meal glucose is below target, reduce pre-meal insulin dose appropriately
	Alcohol has glucose-lowering effects and can mask symptoms of hypoglycemia; consider reducing basal insulin doses when consuming alcohol
Insulin	Basal long-acting + rapid-acting pre-meal insulin combinations less likely to cause hypoglycemia than intermediate acting + regular insulin preparations
	Consider less aggressive correction insulin doses using the '1,800 rule' rather than the '1,500 rule' (i.e. 1,800/total daily dose)
	Avoid repetitive, or stacking of, correction doses
Exercise	Check blood sugars before and during prolonged exercise; snack if necessary
	Consider reducing basal insulin dosage prior to anticipated period of prolonged exercise
	Make adjustments for increased insulin sensitivity for 24 hours after exercise
Treatment	Readily available emergency supplies including sugar tablets, candy, sugar-paste in tube
	Prescription glucagon kits (non-expired) readily available
	People who have regular contact with patient (family members, colleagues, teachers, etc.) need to know signs of hypoglycemia and how to treat it
	Notification of emergency medical services (i.e. 911)
Other prevention strategies	Discussion between patient and physician regarding a period of less intensive glycemic management goals (i.e. relaxed/higher glycated hemoglobin goal, higher blood glucose targets pre-/post-meals, and when calculating correction factor, etc.)
	Scrupulous avoidance of hypoglycemia to restore hypoglycemia awareness
	Medical identification bracelet or necklace indicating that patient has diabetes and takes insulin

to combat iatrogenic hypoglycemia. It is worth noting that other SSRIs, such as sertaline, have been associated with causing hypoglycemia.[49–52] The reason for the discrepancy between fluoxetine's effects and the effects of other SSRIs on hypoglycemia is unknown. Larger clinical studies to investigate the efficacy of fluoxetines as well as its side effects are warranted, but fluoxetine appears to hold some promise as a potential therapy for individuals at high risk for developing hypoglycemia.

The reduced sympathoadrenal response following an episode of antecedent hypoglycemia also has important consequences during sleep. Half of all hypoglycemic events occur at night,[1,18,53] and sleep in itself reduces autonomic responses to hypoglycemia.[54] The combination of reduced defenses against hypoglycemia in patients with diabetes and reduced autonomic responses during sleep drastically increases the risk for nocturnal hypoglycemia. Patients with type 1 diabetes remained asleep 75% of the time during hypoglycemia, whereas patients without diabetes only slept 25% of the time when experiencing hypoglycemia.[55] The reason for the reduced arousal is unknown, but is probably attributed to the blunted sympathoadrenal responses.[14] This reduced arousal may prevent patients from waking up and taking the necessary steps (e.g. ingesting carbohydrates) to counteract hypoglycemia. Hypoglycemia during sleep may go undetected, leading to further drops in blood-glucose levels and possibly resulting in unconsciousness or death.

A study investigated the efficacy of common therapies aimed at reducing nocturnal hypoglycemia: a bedtime snack, bedtime snack plus the α-glucosidase inhibitor acarbose, an uncooked cornstarch bar, or the β_2-adrenergic agonist terbutaline.[56] Only tertbutaline reduced the occurrence of nocturnal hypoglycemia in patients with type 1 diabetes. However, tertbutaline often resulted in morning hyperglycemia. A lower dose of terbutaline than used in that study could possibly prevent nocturnal hypoglycemia without causing morning hyperglycemia.[57]

To date, the only method to reverse HAAF is the scrupulous avoidance of hypoglycemia for two to three weeks. Individuals with HU regained hypoglycemic awareness, and some studies indicate that epinephrine response also returns after two to three weeks of avoiding hypoglycemia.[11–13]

Severe Hypoglycemia—Acute and Long-term Complications

Despite measures to reduce the risk for hypoglycemia, many individuals will experience hypoglycemia and some individuals will experience severe hypoglycemia, which, by definition, requires assistance from another person to restore normal blood glucose levels. The brain needs a constant supply of glucose to maintain its metabolic needs. If glucose delivery to the brain is inadequate, seizures and coma may occur (see *Table 2*). If blood sugar becomes extremely low, massive cerebral failure can occur and may lead to neuronal death.[58,59]

Apart from the acute complication of hypoglycemia, patients often worry about whether multiple episodes of severe hypoglycemia will impair long-term cognitive ability and negatively affect their performance at school or work. In a follow-up of the DCCT study, patients with type 1 diabetes were followed for an average of 18 years and underwent a comprehensive battery of cognitive testing.[60,61] For the study, severe hypoglycemic events were limited to those that led to coma, seizures, or both. The study found no decline in cognitive ability despite multiple episodes of severe hypoglycemia, and no correlation between frequency of severe hypoglycemia and cognitive function was observed.[60,61] Interestingly, a recent study found that recurrent moderate episodes of hypoglycemia may protect the brain against severe hypoglycemia-induced neuronal damage.[62] Thus, moderate episodes of

hypoglycemia may pre-condition the brain and prevent neuronal damage and associated cognitive decline. If extrapolated to the clinical situation, this beneficial adaptation induced by recurrent moderate hypoglycemia could account for the discrepancy between episodes of recurrent hypoglycemia and cognitive decline in intensively treated individuals with diabetes. These data are reassuring, for although hypoglycemia can be acutely dangerous, it does not appear to have long-term adverse effects on cognition in adolescent and adult patients with type 1 diabetes.[60,61]

The effect of hypoglycemia on cognition in infants and very young children is a topic that is under investigation. As the brain is still rapidly developing and thus has higher demand for glucose in young children, a child's brain may be more sensitive to hypoglycemia than an adult brain.[63,64] Hershey found that in children between five and 16 years of age, hypoglycemia was associated with lower spatial intelligence and delayed memory.[65] A study that assessed cognition in children at the time of diagnosis of diabetes and seven years later found that children who experienced hypoglycemic seizures had a decline in verbal abilities and attention.[66] Other studies found deficits in memory[67] and processing speed[66,68] in children with a history of severe hypoglycemia. Although many studies suggest the adult brain to be resistant to cognitive decline due to hypoglycemia, the long-term cognitive effects of hypoglycemia in young children are still controversial.

Management and Prevention of Hypoglycemia

To minimize the frequency and severity of hypoglycemia, several precautions should be considered (see *Table 3*). First, an individualized insulin regime that includes basal insulin and mealtime bolus insulin should be established. Consistent monitoring should be performed before all meals and snacks, before and after exercise, at bedtime, and, if nocturnal hypoglycemia is a concern, occasionally during the middle of the night. The accuracy of carbohydrate intake or 'carb counting' can be improved using reference tables available in cookbooks concerning diabetes and online. During moderate-intensity exercise of 30 minutes or more, a predictable fall in blood sugar can be prevented by a reduction in the insulin dose prior to exercise or consideration of snacks taken during exercise.[12] Following a period of moderate- to high-intensity exercise, a delayed onset of hypoglycemia may occur up to 24 hours later, so adjustments in food and insulin may be warranted.[12] Finally, for individuals with HU who are at high risk for hypoglycemia, hypoglycemia should be avoided for three weeks in order to reverse HAAF.[11–13]

Conclusions

Until a cure for diabetes is found, hypoglycemia will continue to be a major barrier to the achievement of long-term glucose control and will cause recurrent morbidity in individuals with diabetes. Numerous research studies have begun to uncover the mechanisms by which the CNS responds and adapts to hypoglycemia. Understanding these mechanisms will undoubtedly lead to better management and therapies that reduce the risk for hypoglycemia, while still allowing patients to achieve the benefits associated with tight glycemic control. Given this barrier of hypoglycemia for the treatment of diabetes, physicians should discuss hypoglycemia treatment and prevention strategies with their patients taking insulin so that they can have a better chance of achieving their glucose controls goals while avoiding the morbidity and mortality associated with hypoglycemia. ■

1. The Diabetes Control and Complications Trial Research Group, *N Engl J Med*, 1993;329:977–86.
2. The Diabetes Control and Complications Trial/Epidemiology of Diabetes Interventions and Complications Research Group, *N Engl J Med*, 2000;342:381–9.
3. Frier BM, *Diabetes Metab Res Rev*, 2008;24:87–92.
4. Holstein A, Plaschke A, Egberts EH, *Exp Clin Endocrinol Diabetes*, 2003;111:364–9.
5. Leese GP, Wang J, Broomhall J, et al., *Diabetes Care*, 2003;26:1176–80.
6. Suh SW, Hamby AM, Swanson RA, *Glia*, 2007;55:1280–86.
7. Mitrakou A, Ryan C, Veneman T, et al., *Am J Physiol*, 1991;260:E67–E74.
8. De Feo P, Perriello G, De Cosmo S, et al., *Diabetes*, 1986;35:563–9.
9. Fanelli C, Pampanelli S, Epifano L, et al., *Diabetologia*, 1994; 37:797–807.
10. Boyle PJ, Cryer PE, *Am J Physiol*, 1991;260:E395–E402.
11. Cryer PE, *J Clin Invest*, 2006;116:1470–73.
12. Unger J, *Prim Care*, 2007;34:791.
13. Cryer PE, *Diabetes*, 2005; 54:3592–3601.
14. Cryer PE, *N Engl J Med*, 2004;350:2272–9.
15. Gerich JE, Langlois M, Noacco C, et al., *Science*, 1973;182:171–3.
16. Bolli G, De Feo P, Compagnucci P, et al., *Diabetes*, 1983;32:134–41.
17. Segel SA, Paramore DS, Cryer PE, *Diabetes*, 2002;51: 724–33.
18. The Diabetes Control and Complications Trial Research Group, *Diabetes*, 1997;46:271–86.
19. White NH, Skor DA, Cryer PE, et al., *N Engl J Med*, 1983;308:485–91.
20. Gold AE, MacLeod KM, Frier BM, *Diabetes Care*, 1994;17:697–703.
21. Sherwin RS, *Diabetes*, 2008;57:2259–68.
22. Boyle PJ, Nagy RJ, O'Connor AM, et al., *Proc Natl Acad Sci U S A*, 1994;91:9352–6.
23. Boyle PJ, Kempers SF, O'Connor AM, Nagy RJ, *N Engl J Med*, 1995;333:1726–31.
24. de Vries MG, Arseneau LM, Lawson ME, Beverly JL, *Diabetes*, 2003;52:2767–73.
25. Segel SA, Fanelli CG, Dence CS, et al., *Diabetes*, 2001;50: 1911–17.
26. McCall AL, Fixman LB, Fleming N, et al., *Am J Physiol*, 1986;251: E442–E447.
27. Dunn-Meynell AA, Routh VH, Kang L, et al., *Diabetes*, 2002;51:2056–65.
28. Routh VH, *Diabetes Metab Res Rev*, 2003;19:348–56.
29. Choi IY, Seaquist ER, Gruetter R, *J Neurosci Res*, 2003;72:25–32.
30. Gruetter R, *J Neurosci Res*, 2003;74:179–83.
31. Chan O, Zhu W, Ding Y, et al., *Diabetes*, 2006;55:1080–87.
32. Chan O, Lawson M, Zhu W, et al., *Diabetes*, 2007;56:1120–26.
33. Chan O, Cheng H, Herzog R, et al., *Diabetes*, 2008;57:1363–70.
34. Evans ML, McCrimmon RJ, Flanagan DE, et al., *Diabetes*, 2004;53:2542–51.
35. McCrimmon RJ, Evans ML, Fan X, et al., *Diabetes*, 2005;54:3169–74.
36. Gabriely I, Hawkins M, Vilcu C, et al., *Diabetes*, 2002;51:893–900.
37. Gabriely I, Shamoon H, *Diabetes*, 2005;54:609–16.
38. Hassel B, Brathe A, *J Cereb Blood Flow Metab*, 2000;20:327–36.
39. Smith D, Pernet A, Hallett WA, et al., *J Cereb Blood Flow Metab*, 2003; 23:658–64.
40. Pellerin L, *Mol Neurobiol*, 2005;32:59–72.
41. Mason GF, Petersen KF, Lebon V, et al., *Diabetes*, 2006;55:929–34.
42. Davis SN, Dobbins R, Tarumi C, et al., *Am J Physiol*, 1992;263:E688–E695.
43. Davis SN, Colburn C, Dobbins R, et al., *J Clin Invest*, 1995;95: 593–602.
44. Davis SN, Dunham B, Walmsley K, et al., *Am J Physiol*, 1997;272:E567–E575.
45. Fisher SJ, Bruning JC, Lannon S, Kahn CR, *Diabetes*, 2005;54:1447–51.
46. Alquier T, Kawashima J, Tsuji Y, Kahn BB, *Endocrinology*, 2007;148:1367–75.
47. Briscoe VJ, Ertl AC, Tate DB, Davis SN, *Diabetes*, 2008;57:3315–22.
48. Briscoe VJ, Ertl AC, Tate DB, et al., *Diabetes*, 2008;57:2453–60.
49. David S, *Lakartidningen*, 2005;102:582.
50. Pollak PT, Mukherjee SD, Fraser AD, *Ann Pharmacother*, 2001;35:1371–4.
51. Gomez R, Huber J, Tombini G, Barros HM, *Braz J Med Biol Res*, 2001;34:57–64.
52. Takhar J, Williamson P, *Can J Clin Pharmacol*, 1999;6:12–14.
53. The DCCT Research Group, *Am J Med*, 1991; 90:450–59.
54. Jones TW, Porter P, Sherwin RS, et al., *N Engl J Med*, 1998; 338:1657–62.
55. Banarer S, Cryer PE, *Diabetes*, 2003;52:1195–1203.
56. Raju B, Arbelaez AM, Breckenridge SM, Cryer PE, *J Clin Endocrinol Metab*, 2006;91:2087–92.
57. Cooperberg BA, Breckenridge SM, Arbelaez AM, Cryer PE, *Diabetes Care*, 2008;31:2271–2.
58. Auer RN, Kalimo H, Olsson Y, Siesjo BK, *Acta Neuropathol*, 1985;67:25–36.
59. Auer RN, *Forensic Sci Int*, 2004;146: 105–10.
60. Jacobson AM, Musen G, Ryan CM, et al., *N Engl J Med*, 2007;356:1842–52.
61. Musen G, Jacobson AM, Ryan CM, et al., *Diabetes Care*, 2008;31:1933–8.
62. Puente EC, Silverstein J, Daphna-Iken D, et al., *Diabetes*, 2008;57:A5.
63. Amiel SA, *Diabetologia*, 1997;40(Suppl. 2):S62–S68.
64. McCall AL, *Diabetes Care*, 1997;20:1228–30.
65. Perantie DC, Lim A, Wu J, et al., *Pediatr Diabetes*, 2008;9:87–95.
66. Rovet JF, Ehrlich RM, *J Pediatr*, 1999;134:503–6.
67. Kaufman FR, Epport K, Engilman R, Halvorson M, *J Diabetes Complications*, 1999;13:31–8.
68. Northam EA, Anderson PJ, Jacobs R, et al., *Diabetes Care*, 2001;24:1541–6.