

Should minimal blood glucose variability become the gold standard of glycemic control?

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Abstract

The Diabetes Complications and Control Trial (DCCT) established glycosylated hemoglobin (A1C) as the gold standard of glycemic control, with levels $\leq 7\%$ deemed appropriate for reducing the risk of vascular complications. Yet, even when A1Cs were comparable between intensively treated subjects and their conventionally treated counterparts, the latter group experienced a markedly higher risk of progression to retinopathy over time. Our speculative explanation, based on the discovery that hyperglycemia-induced oxidative stress is the chief underlying mechanism of glucose-mediated vascular damage, was that glycemic excursions were of greater frequency and magnitude among conventionally treated patients, who received fewer insulin injections. Subsequent studies correlating the magnitude of oxidative stress with fluctuating levels of glycemia support the hypothesis that glucose variability, considered in combination with A1C, may be a more reliable indicator of blood glucose control and the risk for long-term complications than mean A1C alone.

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1. Introduction

The Diabetes Complications and Control Trial (DCCT) revolutionized diabetes care by confirming the association between hyperglycemia and late diabetic complications (DCCT Research Group, 1993). Glycosylated hemoglobin (A1C) constituted the primary parameter in the study because it provides an integrated measure of glycemic exposure. Considered the “gold standard” of glycemic control, A1C remains the preeminent benchmark of successful therapy, with levels $\leq 7\%$ deemed appropriate for reducing the risk of vascular complications (American Diabetes Association, 2004). Yet, the possibility that more subtle aspects of glucose homeostasis, beyond those represented by A1C, could affect the development or progression of microvascular complications was raised by

the DCCT investigators themselves. In a 1995 report evaluating the association of A1C levels before and during the DCCT with the risk of retinopathy progression in patients receiving either conventional or intensive therapy, the authors surmised that updated mean A1C is “not the most complete expression of the degree of glycemia” and that the risk of complications may be more highly dependent on other factors (DCCT Research Group, 1995). Their statement rested largely on the observation that the risks of retinopathy progression associated with a given level of mean A1C differed significantly between intensively and conventionally treated patients after 5 to 9 years ($P < .01$; Fig. 1). Participants in the intensive treatment group exhibited minimal change in the risk of progression to retinopathy over time, whereas their conventionally treated counterparts experienced a marked escalation of risk, despite comparable A1Cs.

Our speculative explanation for this phenomenon was that there was a greater frequency and magnitude of

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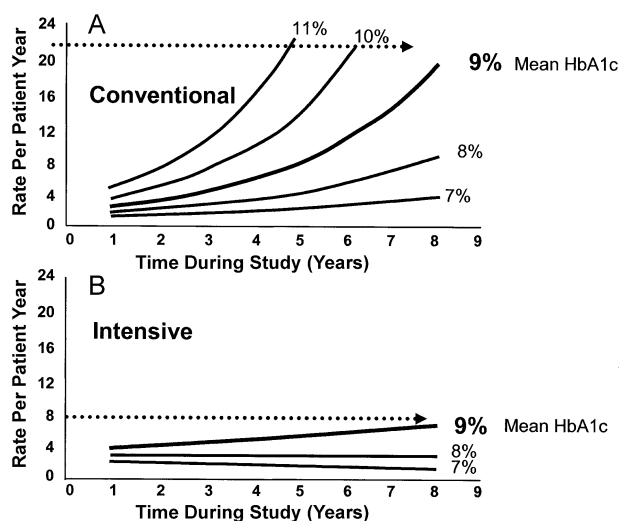


Fig. 1. Absolute risk of sustained retinopathy progression as a function of updated mean A1C (percentage) during the DCCT and the time of follow-up during the study (years), estimated from absolute (Poisson) regression models. (A) Conventional treatment group. (B) Intensive treatment group. Reprinted with permission from DCCT Research Group (1995); © American Diabetes Association.

glycemic excursions in conventionally treated patients, who received fewer insulin injections than did patients in the intensive group. We further postulated that an increased magnitude of glycemic variability would generate more reactive oxygen species (ROS) in complications-prone cells because hyperglycemia-induced oxidative stress, resulting from the overproduction of ROS by the mitochondrial electron-transport chain, is the chief underlying mechanism of glucose-mediated vascular damage (Brownlee, 2001). The ability to quantify oxidative stress in relation to glycemia has provided added insight into the ways in which acute increases in blood glucose, via the production of ROS, affect aspects of physiologic homeostasis. Based on this emerging evidence, we hypothesize that glucose variability, considered in combination with A1C, is a more reliable indicator of blood glucose control and the risk for long-term complications than mean A1C alone.

2. Evidence of the importance of glycemic variability

Although ROS cannot be measured directly, these unstable molecules interact with biological macromolecules such as proteins, lipids, and DNA, to generate numerous oxidative products (Betteridge, 2000). Of these, nitrotyrosine and 8-hydroxydeoxyguanosine (8-OHdG) have been evaluated to determine the extent of vascular damage induced by periodic versus continuous exposure to high glucose (Ceriello, Quagliaro, Catone, et al., 2002; Quagliaro et al., 2003). Examination of downstream targets of ROS, such as cell apoptosis (Risso, Mercuri, Quagliaro, Damante, & Ceriello, 2001), the activation of nuclear factor (NF)- κ B in mononuclear cells (Schiekofer, Andrassy, Chen, et al.,

2003), and cell growth and collagen synthesis in cultured human tubulointerstitial cells (Jones, Saunders, Qi, & Pollock, 1999), has also demonstrated the pathological effects of blood glucose variability.

Quagliaro et al. (2003) investigated the differential effect of variable glucose concentrations versus stable high glucose on high-glucose-induced ROS generation, measured by nitrotyrosine and 8-OHdG levels, and the effects of that oxidative stress on cellular apoptosis, amplifying previous data showing that apoptosis is markedly increased in human umbilical vein endothelial cells (HUVECs) exposed to periodic exaggerations of hyperglycemia (Risso et al., 2001). As predicted, cells cultured in an intermittent glucose condition simulating “real-life” glycemic fluctuations typical of patients with diabetes produced larger amounts of both nitrotyrosine and 8-OHdG compared with constant high and normal glucose conditions. Moreover, exposure of HUVECs to fluctuating glucose concentrations was found to induce a greater increase in the activity of protein kinase C (PKC), a known consequence of hyperglycemia-induced ROS formation, than did stable high glucose (Du, Matsu-mura, Edelstein, et al., 2003; Quagliaro et al., 2003).

In a study of patients with Type 2 diabetes and matched healthy control subjects, fasting nitrotyrosine was significantly increased in diabetic individuals and was further elevated after meals in these patients, but not in the nondiabetic controls (Ceriello et al., 2002). Because nitrotyrosine is a marker of the production of peroxynitrite, a highly reactive free radical derived from superoxide and nitric oxide, it is conceivable that the postprandial state, particularly in people with pronounced hyperglycemia, may have important pathogenic implications.

A comparison of the effects of constant versus alternating hyperglycemia on cultured human proximal tubular cells (PTCs) and cortical fibroblasts (CFs) with respect to cell growth, collagen synthesis, and cytokine secretion revealed enhanced responses following conditions of alternating blood glucose (Jones et al., 1999). Collagen synthesis as a measure of extracellular matrix production was unchanged in PTCs and CFs exposed to constant hyperglycemia, but the presence of intermittent high glucose yielded increases of 29% ($\pm 10\%$, $P < .05$) and 65% ($\pm 28\%$, $P < .01$) in PTCs and CFs, respectively. Secretion of the cytokine TGF- β 1 increased more dramatically in PTCs exposed to alternating (352%) versus constant high glucose (230%); while in CFs, secretion of the cytokine IGFBP-3 was more greatly enhanced (intermittent, 216%; constant, 128%). The authors concluded that variability in glycemic control could be more deleterious to the cells of the tubulointerstitium than constant of high blood glucose, although constant levels of near-normal blood glucose would be least damaging of all.

Finally, diabetic retinal vessels and renal glomeruli contain increased levels of advanced glycation end-products (AGEs). It has been shown that the binding of AGEs, such as carboxymethyllysine (CML), to AGE receptors (RAGE)

activates the pleiotropic transcription factor NF- κ B, causing pathological changes in gene expression (Brownlee, 2001). In a study of peripheral blood mononuclear cells (PBMCs), acute hyperglycemia increased the formation of CML, the activation of ras, p42/44 MAPK phosphorylation, and NF- κ B activity in vivo (Schiekofer et al., 2003). Moreover, isolated PBMCs from healthy volunteers showed increased NF- κ B activation when glucose was raised from 5 to 10 mmol/l, with or without insulin, over 2 h, in the presence of normal AGE levels. This suggests that short-term surges in blood glucose alone are sufficient to mediate NF- κ B activation and signal transduction intrinsic to the development of diabetic complications.

Although additional research is needed to understand why episodic elevations of hyperglycemia magnify certain mechanisms of oxidative stress, preliminary data suggest that protective adaptations induced by constant exposure to hyperglycemia are inadequately activated with intermittent exposure, allowing for more pronounced toxicity (Jones et al., 1999; Risso et al., 2001).

The cumulative effect of chronic high glycemic variability may underlie the phenomenon of “hyperglycemic memory,” whereby hyperglycemia-induced microvascular changes persist or even progress during subsequent periods of normoglycemia (Brownlee, 2001). The applicability of this concept to diabetic patients was confirmed by the results of the Epidemiology of Diabetes Interventions and Complication study showing that the differing effects of conventional or intensive therapy on poststudy progression of microvascular complications and carotid intima-media thickness persisted for 7 and 6 years, respectively, despite the near convergence of A1C levels over time (The Diabetes Control and Complications Trial/Epidemiology of Diabetes Interventions and Complications Research Group, 2002, 2003).

3. Assessing the quality of glycemic control

The results of the aforementioned studies lend weight to our hypothesis that glycemic variability—both postprandial and throughout the day—may be an important but underappreciated mechanism resulting in ROS accumulation and micro- and macrovascular disease acceleration. It is possible that neglecting the prognostic significance of large glycemic variability could lead to the misestimation of the risk for diabetic complications, even if the mean A1C levels fall within or near normal range. Thus, more focused efforts to stabilize glucose variability in diabetic patients may be warranted, even if A1C remains the same.

Currently, diabetes management software that synthesizes data uploaded from blood glucose meters can calculate the standard deviation of blood glucose values (the square root of the variance) to ascertain the quality of glycemic control, that is, whether control is consistent or fraught with extreme variations indicative of suboptimal

disease management (The Diabetes Control and Complications Trial/Epidemiology of Diabetes Interventions and Complications Research Group, 2003). It is possible that, if the hypothesis described herein is correct, the routine use of such software (Hirsch, 2004), both at home and in the clinic, together with A1C monitoring and the strategic administration of rapid-acting insulin analogues, could better modulate glycemia, thereby reducing the incidence and severity of diabetic complications even when A1C levels remain unchanged.

4. Testing the hypothesis

Our hypothesis can be tested from two perspectives. In one study, newly diagnosed patients with Type 2 diabetes would be randomized to one of two treatment arms: The first group would receive one injection of insulin glargine at bedtime, the second, a multiple injection regimen of insulin glargine at bedtime plus mealtime insulin (lispro or aspart) three times per day. Blood glucose would be monitored frequently to determine the degree of glycemic variability between the two groups. The study would be large enough and of sufficient duration to establish diabetic retinopathy as the primary end point, but markers for oxidative stress, as described above, could also be measured for correlation with the degree of glycemic variability within each group and for comparisons between groups.

Alternatively, an analysis of DCCT subjects with identical A1Cs but vastly different retinopathy outcomes could be undertaken to compare the presence and level of specified oxidative markers. Testing for induced antioxidant enzymes, such as superoxide dismutase and glutathione peroxidase, might shed additional light on the complex interplay of glycemic variability, regulation of ROS, and the delay or prevention of diabetic micro- and macrovascular complications.

References

- American Diabetes Association. (2004). Standards of medical care in diabetes. *Diabetes Care*, 27, S15–S35.
- Betteridge, D. J. (2000). What is oxidative stress? *Metabolism*, 49 (Suppl. 1), 3–8.
- Brownlee, M. (2001). Biochemistry and molecular cell biology of diabetic complications. *Nature*, 414, 813–820.
- Ceriello, A., Quagliaro, L., Catone, B., et al. (2002). Role of hyperglycemia in nitrotyrosine postprandial generation. *Diabetes Care*, 25, 1439–1443.
- Du, X., Matsumura, T., Edelstein, D., et al. (2003). Inhibition of GAPDH activity by poly(ADP-ribose) polymerase activates three major pathways of hyperglycemic damage in endothelial cells. *Journal of Clinical Investigation*, 112, 1049–1057.
- Hirsch, I. B. (2004). Blood glucose monitoring technology: translating data into practice. *Endocrine Practice*, 10, 67–76.
- Jones, S. C., Saunders, H. J., Qi, W., & Pollock, C. A. (1999). Intermittent high glucose enhances cell growth and collagen synthesis in cultured human tubulointerstitial cells. *Diabetologia*, 42, 1113–1119.

- Quagliaro, L., Piconi, L., Assalone, R., Martinelli, L., Motz, E., & Ceriello, A. (2003). Intermittent high glucose enhances apoptosis related to oxidative stress in human umbilical vein endothelial cells: The role of protein kinase C and NAD(P)H-Oxidase activation. *Diabetes*, *52*, 2795–2804.
- Risso, A., Mercuri, F., Quagliaro, L., Damante, G., & Ceriello, A. (2001). Intermittent high glucose enhances apoptosis in human umbilical vein endothelial cells in culture. *American Journal of Physiology Endocrinology and Metabolism*, *281*, E924–E930.
- Schiekofer, S., Andrassy, M., Chen, J., Rudofsky, G., Schneider, J., Wendt, T., Stefan, N., Humpert, P., Fritsche, A., Stumvoll, M., Schleicher, E., Hans-Ulrich, H., Nawroth, P. P., & Bierhaus, A. (2003). Acute hyperglycemia causes intracellular formation of CML and activation of ras, p42/44 MAPK, and nuclear factor κ B in PBMCs. *Diabetes*, *52*, 621–633.
- The Diabetes Control and Complications Trial (DCCT) Research Group. (1993). The effect of intensive treatment of diabetes on the development and progression of long-term complications in insulin-dependent diabetes mellitus. *New England Journal of Medicine*, *329*, 977–986.
- The Diabetes Control and Complications Trial (DCCT) Research Group. (1995). The relationship of glycemic exposure (HbA_{1c}) to the risk of development and progression of retinopathy in the Diabetes Control and Complications Trial. *Diabetes*, *44*, 968–983.
- The Diabetes Control and Complications Trial/Epidemiology of Diabetes Interventions and Complications Research Group. (2002). Effect of intensive therapy on the microvascular complications of type 1 diabetes mellitus. *JAMA*, *287*, 2563–2569.
- The Diabetes Control and Complications Trial/Epidemiology of Diabetes Interventions and Complications Research Group. (2003). Intensive diabetes therapy and carotid intima-media thickness in type 1 diabetes mellitus. *New England Journal of Medicine*, *348*, 2294–2303.