

# Is Poor Glycemic Control Associated With Reduced Red Blood Cell Lifespan?

A major assumption in the interpretation of  $HbA_{1c}$  as a measure of glycemic control is that the duration of Hb exposure to glucose does not vary among patients who are hematologically normal. In this issue of *Diabetes Care*, Virtue et al. (1) use an appealingly simple method to quantitate erythrocyte lifespan (2,3) and report an inverse correlation with glycohemoglobin concentration in patients with diabetes. They interpret this as a reduced erythrocyte lifespan at higher blood glucose concentrations. The calculation of erythrocyte lifespan depends on Hb concentration and the amount of carbon monoxide (CO) in exhaled air and in atmospheric air collected at the subject's home just after awakening. The principles behind the method are that heme catabolism is the only endogenous source of CO and that erythrocytes removed from the circulation are the major source of heme. However, there are multiple assumptions behind the methodology, which may not be met, in the complicated scenario of diabetes. These all come together in Eq. 1 of Virtue et al. (see [1] for the definition of terms)

Erythrocyte lifespan =

$$\frac{[Hb](22,400)(\text{blood volume})}{0.7(\text{endogenous } P_{co}(64,400)(1,440)(\text{alveolar ventilation})}$$

which determines erythrocyte lifespan as the inverse of the CO excretion rate, estimating from CO the amount of heme metabolized, and then the amounts of Hb and, finally, red blood cells removed. Heme is a component not only of Hb but also of cytochromes and muscle myoglobin, with cytochromes being ubiquitous but particularly enriched in liver. The Hb concentration depends not only on erythrocyte mass but also on plasma volume; hence, it is sensitive to variations in hydration and in renal, liver, and cardiac function. CO concentration in expired air is determined by not only endogenous production and ventilation rate but also by environmental exposure, predominantly from cigarette smoking (including secondary smoke) and fossil fuels. The

authors assume that the percentage of heme turnover accounted for by erythrocytes is constant at 70% and use the absence of both liver disease and use of known drug inducers of cytochromes as evidence for the validity of that assumption. While not stated in the article, it is also assumed that variations in exercise intensity or muscle trauma or even muscle mass on muscle myoglobin heme turnover are not important. The parameters the authors actually measure are the CO tensions and the [Hb] because the authors assume that variation in minute ventilation cancels out with variation in blood volume because both are related to weight as long as the subject is currently a nonsmoker who does not appear to have pulmonary disease. This assumption comes into question, however, by an opposite trend that is likely to appear in this population, namely that increasing degrees of obesity raise the chance of undiagnosed restrictive lung disease. It would be of interest to know the relative body weights of the study subjects. We would point out that variations in plasma volume would act in one direction on [Hb] and in the opposite direction on blood volume, and these would tend to cancel each other out. Other screening criteria included normal blood counts and liver function, normal serum creatinine in 21 of 23 subjects, no clinical evidence of heart failure, dehydration, and alcoholism, and indirectly, by studying only men, the exclusion of subjects with blood loss from menstruation. The authors argue that there is an ~40% difference in erythrocyte lifespan between those with high versus low GHb and that there is not likely to be that great a change in any one of these parameters without clinical evidence of disease, which would have triggered an exclusion of a potential subject. This is not entirely reassuring in a population of 40- to 77-year-old men with diabetes, in whom the probability of multiple subclinical diseases is high.

What is the evidence that this technique is valid? First, the authors present

evidence that the underlying component measurements are reproducible, which is important. There is strong support for the principle of the technique in the demonstration (Fig. 2A) of a significant negative correlation of erythrocyte lifespan calculated by the CO technique with reticulocyte count in patients with normal [Hb]. What is not so clear is the validation of the precise quantitation of the method, given the number of assumptions and the population examined. In the original description of the technique (2), the authors demonstrate a less-than-perfect correlation between CO-erythrocyte lifespan and the standard  $^{51}\text{Cr}$  technique. Unfortunately, rather than validate the CO method against the gold standard of  $^{51}\text{Cr}$  survivals, they invoked the limitations of the  $^{51}\text{Cr}$  method to explain the discrepancies. The limitations of the chromium technique, primarily the leaching of the chromium label from the red blood cells, are known and can be mathematically corrected. With such an approach, two of us (R.S.F. and C.H.J.) and others (4,5) have reported a close relationship between erythrocyte lifespan determined using  $^{51}\text{Cr}$  and biotin labels. The biotin label overcomes many of the disadvantages of  $^{51}\text{Cr}$ . It is nonradioactive and provides a stable label for the entire erythrocyte lifespan. The biotin technique requires fewer assumptions than the CO method. However, the latter can be applied to larger numbers of subjects and can be used to perform serial measurements over time. Clearly, the biotin label offers the opportunity for more precise validation of the quantitative aspects of the CO-based results, giving greater confidence in the absolute values of the lifespans.

So, what does all this have to do with diabetes? Assuming the validity of the CO methodology, the authors interpret their data as showing that erythrocyte lifespan is shortened by long-term glycemic control, as reflected in  $HbA_{1c}$ . This idea is also supported by the original studies of Peterson et al. (6), which showed a modest but

consistent increase in  $^{51}\text{Cr}$  erythrocyte half-life after the establishment of tight glycemic control compared with the same patients studied in poor control. If higher levels of GHb are associated with shorter erythrocyte lifespan, the implication is that the difference in glycemic control between two levels of achieved  $\text{HbA}_{1c}$  is actually greater than our current data would lead us to believe, with the caveats we have mentioned about the magnitude of the effect.

What about implications for erythrocyte disorders in patients with diabetes if these findings are corroborated? It is plausible that if hyperglycemia results in a reduction in erythrocyte lifespan within the normal range, this could be a stress on the erythron (erythroid marrow and circulating erythrocytes), which may be unmasked by other erythrocyte diseases. It may have an implication for how long transfused erythrocytes survive in patients with poorly controlled diabetes compared with those in better control or without diabetes. This suggests that tighter glycemic control might offer hematologic benefit to diabetic patients undergoing chemotherapy or having a chronic transfusion or erythropoietin requirement.

The finding that  $\text{HbA}_{1c}$  varies with erythrocyte lifespan reported here is yet one more piece of evidence that there are more factors influencing the relationship between glycated hemoglobins and glycemic control than we have conventionally taken into account. Further evidence along those lines is emerging from a number of directions. Greater differences in  $\text{HbA}_{1c}$  between fraternal than identical nondiabetic twins argue for the role of ge-

netic factors apart from glycemic control (7). McCarter et al. (8) and we (9) have shown that elevation of  $\text{HbA}_{1c}$  out of proportion to other measures of glycemic control, either fingerstick glucoses or fructosamine, respectively, is associated with higher frequencies of complications. We have taken  $\text{HbA}_{1c}$  at face value for a long time, and it is now time to examine factors affecting it a little more closely. With that, we should improve our ability to predict the risk of complications and tailor therapy more precisely for individuals with diabetes.

ROBERT M. COHEN, MD<sup>1,2</sup>  
ROBERT S. FRANCO, PHD<sup>3,4</sup>  
CLINTON H. JOINER, MD, PHD<sup>4,5</sup>

From the <sup>1</sup>Division of Endocrinology, Department of Medicine, University of Cincinnati Medical Center, Cincinnati, Ohio; <sup>2</sup>Medical Service, Cincinnati Veterans Affairs Medical Center, Cincinnati, Ohio; the <sup>3</sup>Division of Hematology/Oncology, Department of Medicine, University of Cincinnati Medical Center, Cincinnati, Ohio; the <sup>4</sup>Cincinnati Comprehensive Sickle Cell Center, Cincinnati Children's Hospital Research Foundation, Cincinnati, Ohio; and the <sup>5</sup>Department of Pediatrics, University of Cincinnati Medical Center, Cincinnati, Ohio.

Address correspondence to Robert M. Cohen, MD, Division of Endocrinology/Metabolism, P.O. Box 670547, Vontz Center for Molecular Studies, 3125 Eden Ave., University of Cincinnati Medical Center, Cincinnati, OH 45267-0547. E-mail: robert.cohen@uc.edu.

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