The following is the abstract of the article discussed in the subsequent letter:

Varma, Shubha, Brajesh K. Lal, Ruifang Zheng, Jerome W. Breslin, Satoshi Saito, Peter J. Pappas, Robert W. Hobson II, and Walter N. Durán. Hyperglycemia alters PI3k and Akt signaling and leads to endothelial cell proliferative dysfunction. Am J Physiol Heart Circ Physiol 289: H1744–H1751, 2005. First published June 17, 2005; doi:10.1152/ajpheart.01088.2004—Diabetes mellitus is a major risk factor for the development of vascular complications. We hypothesized that hyperglycemia decreases endothelial cell (EC) proliferation and survival via phosphatidylinositol 3-kinase (PI3k) and Akt signaling pathways. We cultured human umbilical vein ECs (HUVEC) in 5, 20, or 40 mM D-glucose. Cells grown in 5, 20, and 40 mM mannitol served as a control for osmotic effects. We measured EC proliferation for up to 15 days. We assessed apoptosis by annexin V and propidium iodide staining and flow cytometry, analyzed cell lysates obtained on culture day 8 for total and phosphorylated PI3k and Akt by Western blot analysis, and measured Akt kinase activity using a GSK fusion protein.

HUEC proliferation was also tested in the presence of pharmacological inhibitors of PI3k-Akt (wortmannin and LY294002) and after transfection with a constitutively active Akt mutant. ECs in media containing 5 mM D-glucose (control) exhibited log-phase growth on days 7–10. D-Glucose at 20 and 40 mM significantly decreased proliferation versus control (P < 0.05 for both), whereas mannitol did not impair EC proliferation. Apoptosis increased significantly in HUEC exposed to 40 mM D-glucose. D-Glucose at 40 mM significantly decreased tyrosine-phosphorylated PI3k, threonine 308-phosphorylated-Akt, and Akt activity relative to control 5 mM D-glucose. Pharmacological inhibition of PI3k-Akt resulted in a dose-dependent decrease in EC proliferation. Transfection with a constitutively active Akt mutant protected ECs by enhancing proliferation when grown in 20 and 40 mM D-glucose. We conclude that D-glucose regulates Akt signaling through threonine phosphorylation of Akt and that hyperglycemia-impaired PI3k-Akt signaling may promote EC proliferative dysfunction in diabetes.

Does Hyperglycemia Reduce Proliferation or Increase Apoptosis?

To the Editor: Varma et al. (4) present an interesting analysis of the effects of hyperglycemia on cultured human endothelial cells. They find that in the presence of 20 or 40 mM glucose, the growth of endothelial cells is much reduced, the activity of the phosphatidylinositol 3-kinase (PI3k)/Akt pathway is decreased, and restoration of Akt activity restores the rate of cell growth. They interpret their data as demonstrating that hyperglycemia decreases proliferation by decreasing activity of the PI3k/Akt pathway. Although we find their data persuasive and convincing, we believe it more strongly supports an alternative explanation: that an increase in apoptosis is the critical effect of hyperglycemia in this system. The apoptotic program is relatively rapid and may be completed within 8 h in cells in vitro (1), and although it is true that, in vitro, these cell remnants will not be phagocytosed, they may detach from the tissue culture dish and be lost to subsequent analysis. Thus even a relatively small measured level of apoptosis might reflect a substantial rate of cell death. For example, Fig. 1 illustrates two hypothetical cell populations with different rates of cell loss due to apoptosis at each division. If we suppose that the 4.81% apoptotic cells observed by Varma et al. (4) reflects an actual rate of cell loss of 3 times that amount over the course of 1 day, then after fewer than 10 divisions, 50% fewer cells remain compared with the control population.

As alluded to by Varma et al. (4), any difference in cell numbers may be due to either a reduced proliferation rate or increased cell death. In this regard, it is important to make the distinction between cell growth (i.e., increase in total cell numbers) and cell proliferation (i.e., the rate of cell division). Because proliferation rate was not directly examined, no direct conclusion can be made about the effect of hyperglycemia on proliferation. On the other hand, levels of cell death were measured and found to increase significantly with increasing glucose (4.81% apoptotic in 40 mM glucose vs. 2.35% in control, P < 0.01), a difference of 2.46%. Indeed, a recent publication (3) found that apoptosis of human umbilical vein endothelial cells is increased in hyperglycemic culture and that this can be prevented by overexpression of Akt.

We do not discount the possibility that hyperglycemia may limit cell growth by inhibiting proliferation as well as increasing apoptosis. The PI3k/Akt pathway has been implicated in both proliferation and antiapoptosis in several cell types of the myocardium, including cardiomyocytes and endothelial cells (2). However, it is not possible to assess the relative involvement of proliferation and apoptosis by counting cell number alone. Counting the frequency of mitoses in cells grown in different culture conditions might be one simple method to address this important question. We would welcome the comments of the authors on the points raised above.

REFERENCES


Sean M. Davidson
Derek M. Yellon
The Hatter Cardiovascular Institute
Royal Free and University College Medical School
Department of Medicine
67 Chenes Mews
University College Hospital
London WC1E 6HX, United Kingdom
e-mail: s.davidson@ucl.ac.uk
REPLY

To the Editor: We thank Drs. S. M. Davidson and D. M. Yellon for their review of our published data and are pleased to respond to their comments. With regard to the distinction between cell growth and proliferation, we are aware of the ample menu of definitions offered in the literature, particularly in the field of oncology, regarding the terms growth, proliferation, and proliferation rate. As we stated in Varma et al. (1), any difference in cell numbers may be due to either a reduced proliferation rate or increased cell death. Therefore, we submit that our original interpretation and their comments are both tenable. We determined cell count/well as a function of time for a set of experiments as shown on Fig. 1 of Varma et al. (1), but all subsequent data (Akt phosphorylation, etc.) were obtained on day 8 (midlog phase) of exposure to different concentrations of glucose. Thus, although we know the slope of a particular data set, we did not evaluate changes in total and phosphorylated Akt, etc., as a function of time.

Because the timing of the initiation of apoptosis and the rate at which it persisted in each cell division cycle are unknown, as is the extent of the contribution of necrosis to loss of cells, the occurrence of the hypothetical situation described in Fig. 1 of the Letter to the Editor by Davidson and Yellon is unclear but possible. Regardless of whether Akt works preferentially via proliferation or via apoptosis, it is clear that this kinase plays a major role in glucose-induced endothelial dysfunction. As we demonstrated (1), transfection of Akt+/+ rescues these cells from the deleterious effects of 20 and 40 mM glucose.

We appreciate the interest expressed by Drs. Davidson and Yellon in our work.

REFERENCES


Brajesh K. Lal
Shubha Varma
Ruifang Zheng
Peter J. Pappas
Walter N. Durán
Department of Pharmacology and Physiology
and Department of Surgery
University of Medicine and Dentistry of New Jersey
New Jersey Medical School
Newark, New Jersey
e-mail: duran@umdnj.edu