Diabetes is the epidemic of the 21st century. Rare in the past, diabetes has grown into an increasingly common disease both in developed countries and most recently also in the third world. The most important factor for this unforeseen trend appears to be the increase in body weight around the world attributable to the changes in lifestyle over the last decades. It is likely that the increasing prevalence of diabetes will greatly affect the cardiovascular disease burden in the future. Although the morbidity and mortality of cardiovascular disease has fallen over the last 3 decades, this trend may flatten or even reverse.

Thus, a better understanding of the consequences of diabetes in the vasculature and the heart is of great importance. Indeed, diabetes markedly affects the function of the cardiovascular system, both in the microcirculation as well as in large conduit arteries supplying vital organs such as the heart, brain, and kidney. As a consequence, diabetes surpasses other conditions such as dyslipidemia and hypertension as a risk predictor for myocardial infarction, stroke, and renal failure; in fact, because of its severe prognosis, diabetes may be considered the cancer of the vasculature.

For vascular homeostasis, endothelial cells are of utmost importance. Indeed, these cells produce a variety of mediators, surface proteins, and autacoids involved in vasomotion, coagulation, and inflammation. In humans and animal models of diabetes in vivo as well as in endothelial cells in culture exposed to high glucose concentrations, marked functional changes are observed. A major mediator of endothelial function is nitric oxide (NO), which is synthesized from L-arginine via endothelial nitric oxide synthase (eNOS). In diabetes, endothelium-dependent relaxations are reduced both in humans and experimental animals. Surprisingly, though, early studies found an increased rather than a reduced expression of eNOS in human aortic endothelial cells in culture treated with high glucose and, later, also in arteries obtained from diabetic animals. Although this initially seemed paradoxical, it soon became clear that biologically active NO is chemically inactivated under these conditions because of the production of reactive oxygen species (ROS). ROS and, in particular, superoxide (O2\(^{-}\)) rapidly interact with NO to form peroxynitrite, a highly reactive molecule that leads to nitrosylation of vital cellular proteins, among others prostacyclin synthase and superoxide dismutase, as well as DNA damage (Figure). Thus, endothelial dysfunction in diabetes is most likely related to these biochemical alterations: sweet and sour seems to underlie early diabetic vascular disease.

The molecular and cellular mechanisms involved in glucose-induced endothelial dysfunction have been partially clarified over the last decade. It appears that high glucose concentrations activate protein kinase C and, specifically, its \(\beta_2\) isoform. Because glucose, but not mannitol, exerts the above mentioned effects on NO and ROS and also specifically activates protein kinase CB2 in endothelial cells, these effects appear to be unrelated to changes in osmolality, but rather reflect activation of distinct cellular pathways (Figure). Protein kinase CB2 has been implicated in the regulation and activation of membrane-associated NAD(P)H-dependent oxidases and subsequent production of superoxide anions and hence may initiate oxidative stress, which is a hallmark of diabetic vascular dysfunction.

More recently, another pathway possibly involved in this context has been characterized. Indeed, p66\(\text{shc}\) is a mitochondrial adaptor protein involved in ROS metabolism and apoptosis. Interestingly, genetic deletion of p66\(\text{shc}\) in mice prolongs life span by approximately one-third, bluntts cellular ROS production, reduces the cellular susceptibility to ROS, and prevents age-dependent endothelial dysfunction. Most strikingly, experiments in p66 knockout mice with streptozotocin-induced diabetes have revealed that endothelium-dependent relaxations in the aorta are preserved in spite of markedly elevated glucose levels in these animals. Concomitantly, in contrast to their wild-type littermates, ROS formation is not upregulated in diabetic p66\(^{-}\) mice, nor is ONOO\(^{-}\) formation increased. As a consequence, the amount of nitrosylated proteins remains at a normal level. Thus, these results suggest that glucose may not only activate protein kinase CB2 but also (possibly via the latter enzyme) other pathways such as p66\(\text{shc}\) to increase ROS production under diabetic conditions. ROS are known to activate transcription factors, such as nuclear factor \(\kappa\)B, that regulate inflammatory and procoagulant genes (Figure). These molecular events may well explain vascular dysfunction, inflammation, and disease associated with diabetes in patients.

In this issue of Circulation Research, Romero et al expand on these concepts by their study on the effects of diabetes on arginase activity. L-Arginine is not only a substrate for eNOS but also for arginase. Arginase exists in 2 isoforms: arginase I is located in the cytoplasm and expressed abun-
dantly in the liver as part of the urea cycle, whereas arginase II is of mitochondrial origin and primarily expressed in the kidney. Arginase competes therefore with eNOS for L-arginine availability. Interestingly, upregulation of the enzyme has been described in diabetes. Thus, endothelial dysfunction in diabetes may be caused, at least in part, by reduced L-arginine availability for eNOS. In streptozotocin-induced diabetes of the mouse, Romero et al first confirmed reduced endothelium-dependent vasodilation as well as increased production of ROS in this model. Importantly, however, they demonstrate, for the first time, an increased arginase I activity and expression in the aorta and liver of mice treated with streptozotocin. Furthermore, acute treatment of diabetic coronary arteries with an arginase inhibitor reversed the impaired endothelium-dependent relaxation to acetylcholine, confirming that this cytosolic enzyme reduces substrate availability for eNOS. In streptozotocin-induced diabetes of the mouse, Romero et al first confirmed reduced endothelium-dependent vasodilation as well as increased production of ROS in this model. Importantly, however, they demonstrate, for the first time, an increased arginase I activity and expression in the aorta and liver of mice treated with streptozotocin. Furthermore, acute treatment of diabetic coronary arteries with an arginase inhibitor reversed the impaired endothelium-dependent relaxation to acetylcholine, confirming that this cytosolic enzyme reduces substrate availability for eNOS and in turn contributes to endothelial dysfunction in this model of diabetes. Similarly, bovine aortic endothelial cells treated with glucose exhibited an augmented arginase activity, suggesting that plasma glucose levels are the major mediator of this effect. This conclusion is convincingly supported by experiments using arginase I small interfering RNA, which prevented the rise in arginase activity in endothelial cells treated with high glucose concentrations.

The molecular mechanism of glucose-induced upregulation of arginase activity appears to involve small G proteins. Indeed, the Rho kinase inhibitor Y-27632 as well as a HMG–coenzyme reductase inhibitor (statin) blunted the up-regulation of the enzyme as well as ROS production under these conditions. Statins are known to inhibit the Rho/Rock pathway, and they reduce vascular events in patients with diabetes as demonstrated in many clinical trials.13,14 This set of experiments therefore further substantiates evidence that the Rho/Rock pathway may contribute to the vascular protective effects of statins. Besides in arginase I, this signal transduction mechanism is also involved in the regulation of the expression of eNOS, tissue factor, and other gene products. However, it is likely that other pathways also contribute to arginase I upregulation in diabetes. Indeed, cytokines and also ROS themselves have been shown to increase arginase I expression and/or activity.15

The finding of increased arginase I activity in diabetes may limit other therapeutic approaches proposed for early endothelial dysfunction such as oral L-arginine supplementation. Although dietary L-arginine supplementation has been shown to exert vascular protective effects in certain clinical settings,16 this approach is unlikely to be effective in diabetes, if the results of this study can be confirmed in patients in vivo. In fact, the findings of Romero et al may provide a possible explanation for the unexpected neutral or even adverse effects of oral L-arginine in some clinical studies, in particular in patients with coronary artery disease and infarction.17 If, on the other hand, ROS production turns out to be the primary event in the upregulation of arginase I, pathways involved in oxidative stress may represent more promising targets to prevent endothelial dysfunction in diabetes. Indeed, activa-
tion of NAD(P)H-dependent oxidases and their subsequent O2− production is regulated by angiotensin II, and inhibitors of angiotensin converting enzyme as well as angiotensin type 1 receptors are particularly effective in diabetic patients. However, the suppression of oxidative stress, proteinuria, and clinical events exerted by such compounds is far from optimal. Thus, novel molecules such as p66shc, which experimentally appear to be heavily involved on diabetic vascular disease as well as cardiomyopathy should be further pursued within this context.

Thus, as we look at sweet and sour in the vessel wall, we proceed further toward the understanding of the molecular and cellular mechanisms of diabetic vascular disease that account for the majority of morbidity and mortality in this increasing patient population. Such concepts may set the basis for future and more effective treatment of these patients that are at high risk for major adverse cardiovascular events.

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Disclosures

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References
