

inside **blood** commentary

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● ● ● THROMBOSIS AND HEMOSTASIS

Comment on Xiang et al, page 3377

microRNA represses macromolecule

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In this issue of *Blood*, Xiang et al identify a novel mechanism, involving activation of the polyol pathway and repression of microRNA-24 (miR-24), through which hyperglycemia augments von Willebrand factor (VWF) expression and secretion.¹

Hyperglycemia alters glucose metabolism profoundly. Increased flux of glucose into the polyol pathway, when the capacity of the glycolytic pathway is saturated, is one of the major metabolic changes that has been associated with the development of diabetic complications.² The enzymes aldose reductase and sorbitol dehydrogenase generate sorbitol and fructose in processes that use nicotinamide adenosine dinucleotide phosphate (NADPH) and nicotinamide adenine dinucleotide (NAD⁺) as cofactors. As increasing amounts of glucose enter the polyol pathway, 2 major mechanisms lead to an increase in oxidant

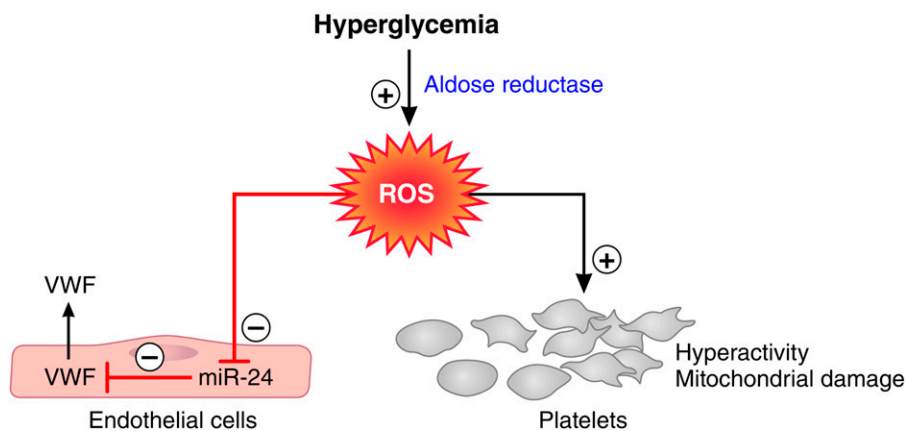
stress: (1) the augmented formation of NADH leads to the production of reactive oxygen species (ROS) by NADH oxidase, and (2) the depletion of NADPH causes reduction of cytosolic glutathione levels, impairing the antioxidant defense system of the cell. Xiang et al discover that polyol pathway-mediated oxidant stress represses miR-24, which leads to elevated plasma VWF concentrations in hyperglycemia (see figure).¹ Previous work conducted in the same laboratory showed that polyol-mediated oxidant stress also contributes to platelet hyperactivity and platelet

mitochondrial damage in diabetic patients (see figure).^{3,4}

Mature VWF mediates platelet adhesion and aggregation and stabilizes blood coagulation factor VIII.⁵ Elevated VWF levels have been associated with arterial thrombosis⁶ and poor glycemic control.⁷ However, whether and how high glucose might cause upregulation of VWF remain unknown.

Xiang et al hypothesized that miRNAs might be involved in the regulation of VWF and screened 29 candidate miRNAs in reporter gene assays using either the human or the mouse VWF 3' untranslated region (3'UTR). Although miR-24 was not the only hit, it was the miRNA with the most pronounced effects on both mouse and human VWF 3'UTR activity. As expected, circulating miR-24 levels were reduced and plasma VWF levels elevated in patients with type 1 and type 2 diabetes compared with age- and sex-matched healthy controls.^{7,8} Importantly, mouse biology recapitulated the observations in humans faithfully. Three diabetic mouse models showed decreased miR-24 and increased VWF levels.

Xiang et al exploited the conservation of miR-24 to conduct a series of loss- and gain-of-function experiments in mice using anti-miRNAs and miRNA mimics. These experiments revealed the causal links among repression of miR-24, increase in VWF expression, and propensity for thrombosis. Experiments in cells showed that miR-24 regulated multiple events in VWF biosynthesis, maturation, and secretion, which may all contribute to the increase in VWF plasma levels when miR-24 is repressed. Repression of miR-24 by high glucose was reversed by an inhibitor of the rate-limiting enzyme in the polyol pathway: aldose reductase. Experiments with the aldose reductase inhibitor also revealed that c-Myc was activated by glucose flux through the polyol pathway and knockdown of c-Myc in turn increased miR-24 levels with remarkable specificity.



Aldose reductase-mediated effects of hyperglycemia on the coagulation system. Flux of glucose via aldose reductase into the polyol pathway generates ROS. ROS formation depresses miR-24, which releases this miRNA's block on VWF secretion and results in elevated VWF plasma levels. Aldose reductase-mediated ROS formation also causes platelet hyperactivity and mitochondrial damage. Red lines, negative regulation; black arrows, positive regulation. Professional illustration by Patrick Lane, ScEYence Studios.

Fitness deficits in long-term ALL survivors

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In this issue of *Blood*, Ness et al report that, despite the omission of prophylactic cranial radiotherapy (CRT) in the treatment of acute lymphoblastic leukemia (ALL), adult survivors of childhood ALL remain at risk for impaired fitness, body composition, and energy balance.¹

These findings reveal a mechanism for the elevated levels of high-molecular-weight VWF observed in diabetic patients and possibly also for the increased risk of thrombotic events. Further work will be required to assess whether miR-24 and VWF levels can be manipulated in humans (eg, with miR-24 mimics) and whether such interventions have potential as antithrombotic therapies in patients with diabetes mellitus. Targeting the polyol pathway seems conceptually attractive because of the coincident activities on platelets^{3,4} and VWF¹ (see figure), although it is not the only source of oxidant stress in diabetes. Indeed, several aldose reductase inhibitors have been developed and undergone clinical testing primarily for diabetic neuropathy, but there remains uncertainty regarding their efficacy and safety. One of the problems in diabetic neuropathy is the imprecision of biomarkers and measures of efficacy. However, the impact of aldose reductase inhibitors on VWF concentrations and platelet function may be more quantitatively assessable.

These novel results on the molecular mechanisms contributing to thrombosis in diabetic patients may lead to new opportunities for therapeutic intervention.

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Survival rates for most childhood cancers have increased dramatically over the last 40 years, with >80% of pediatric patients diagnosed with ALL becoming 5-year survivors.² Unfortunately, healthspan is not keeping pace, and the challenge now is to reduce treatment-related chronic health problems that occur in ~70% of long-term survivors.³ Childhood cancer survivors are twice as likely to be obese compared with their siblings,⁴ and adult survivors of childhood ALL are at an increased risk of adiposity, dyslipidemia, diabetes, and cardiovascular disease.⁵⁻⁷ Furthermore, many studies have reported additive long-term detrimental effects of receiving prophylactic CRT during primary cancer treatment.⁵⁻⁷ Once a standard treatment of ALL, CRT has all but been replaced with intrathecal and systemic chemotherapy to reduce radiation-associated late complications such as cancer recurrence, cognitive decline, and metabolic risk.² However, for the sake of future childhood ALL survivors, it is important to know if omitting CRT has alleviated long-term health problems associated with excess adiposity and poor cardiorespiratory fitness.

Ness et al determined the impact of CRT on the well-characterized decrements in body composition, energy balance, and physical fitness in adult survivors of childhood ALL. All participants (n = 365) were >18 years of age, at least 10 years from diagnosis, had a mean survival time of 21.9 years, and were previously treated for ALL at St. Jude Children's Research Hospital (SJCRC) when <18 years of age. A noncancer comparison group that was age, sex, and race matched to the study participants was recruited from SJCRC visitors. The study recruited 87.7% of all eligible patients, and the "gold standard" techniques of indirect calorimetry, dual x-ray

absorptiometry, and doubly labeled water were used to assess physical fitness, body composition, and energy expenditure, respectively.

Survivors exposed to CRT had higher body mass index and percentage of body fat scores than survivors not exposed to CRT, but only male non-CRT survivors had less favorable body composition scores than the comparative group. Body-mass-adjusted total daily energy expenditure and activity energy expenditure were also lower in CRT compared with non-CRT survivors. Moreover, cardiorespiratory fitness (peak oxygen uptake) and muscular strength (handgrip and quadriceps) and endurance (quadriceps) were lower in CRT-exposed survivors compared with non-CRT survivors. Measures of cardiorespiratory fitness, peripheral sensory integrity, proximal muscle strength, and flexibility were markedly lower in ALL survivors than in the comparative group. Although CRT exposed survivors had the lowest mean values for most physical fitness measures, non-CRT-exposed survivors still had large fitness deficits relative to the comparison group, particularly for proximal muscle strength and aerobic exercise capacity. Ness et al also reported for the first time that cumulative exposure to asparaginase and glucocorticoids was associated with impaired flexibility and weak hand strength, respectively. They also confirmed previously reported associations between vincristine dose and peripheral neuropathy and between intrathecal methotrexate dose and balance problems.

It is intuitive to speculate that the detrimental effects of CRT exposure on body composition and fitness are due to treatment-related deficiencies in hypothalamic pituitary hormones. Another recent study