Endothelial Iron Status In Vivo

In cultured endothelial cells, iron status can be readily manipulated through the use of iron chelators or supplemental iron, or by altering the essential fatty acid composition of the culture medium. Use of these methods has identified several important effects of iron in endothelial activation and oxidative injury. But the in vitro approach does not define endothelial iron status in vivo, in particular what might represent a physiologically optimal range of endothelial iron concentrations. Iron status parameters such as serum ferritin are imperfect measures of total body iron stores and are likely to be even less adequate in assessing endothelial iron status. Iron status parameters such as serum ferritin represent a physiologically optimal range of endothelial iron concentrations. This view is related to the traditional acceptance of ranges of added iron shown, for example, to markedly influence production of adhesion molecules in cultured endothelial cells.

It is not clear whether iron content in freshly harvested HUVEC, or endothelial cells from other sources for that matter, accurately reflects iron content in vivo, or, perhaps is produced as an artifact of the conditions of harvesting. If the order of magnitude greater iron content of freshly prepared cells approximates in vivo conditions, might this higher level be typical of endothelial iron status in vivo? If so, the in vitro work seems to suggest that endothelial cells could be quite sensitive to oxidative injury in vivo. However, there may be tissue specificities with respect to endothelial iron status. HUVEC are derived from umbilical vein, and as such could have endothelial iron levels higher than those encountered in other tissues at the time of harvesting because of regulatory factors involved in iron transfer from mother to fetus. Newborns, especially premature newborns, have increased susceptibility to oxidative injury. It has been proposed that this is attributable in part to features of iron metabolism in the neonatal period. Even term newborns typically undergo a brief period of high serum ferritin and transferrin saturation shortly after birth. Endothelial cells harvested from other tissues in adult subjects may initially have higher than optimal iron contents because of the presence of storage iron in the donor subjects favoring increased susceptibility to oxidative injury.

The findings of Zheng et al suggest that flow-mediated dilation could be a new tool for monitoring endothelial iron status in vivo. Additional work is needed to confirm this possibility, including assessment of the effects of iron depletion on endothelium-independent vasodilation. Flow-mediated dilation may increase inversely with endothelial iron but the relationship may not be linear. Some improvement in flow-mediated dilation has been documented in patients with hemochromatosis after partial phlebotomy treatment, but the most prominent effects might be seen over a range of iron contents near iron deficiency. Interestingly, endothelium-independent dilation was unaffected by initial

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Editorial

Stored Iron and Vascular Reactivity

Jerome L. Sullivan

Zheng et al report that volunteer blood donors with a high donation frequency have significantly greater flow-mediated dilation than low frequency donors. Iron status among the frequent donors approximated a state of iron depletion by assessment of conventional iron markers. The study provides important support for the “iron hypothesis,” which suggests a protective effect of iron depletion, i.e., the absence of storage iron without anemia, against ischemic heart disease. The blood donor findings suggest a new direction for the study of endothelial iron status in vivo to complement a growing body of work on iron in cultured endothelial cells. In addition, the finding of lower serum nitrotyrosine in frequent donors is consistent with the hypothesis that myeloperoxidase, a powerful emerging cardiovascular risk factor, is modifiable by manipulation of iron status.
treatment in patients with hemochromatosis. This suggests that iron impairs flow-mediated dilation largely by modulating endothelial function. Serum ferritin level does not necessarily correlate well with maximal effects of iron loss near the lower end of the iron load spectrum. For example, in hepatitis C, iron reduction therapy can lower the level of serum aminotransferases. It has been reported that serum aminotransferase levels are a better guide to therapy because they continue to decrease with additional iron removal even after serum ferritin has reached its minimum value. Analogously, flow-mediated dilation could be a better guide than serum ferritin to achieving desired end points in endothelial iron status.

**Does Iron Depletion Improve Vascular Function by Decreasing Myeloperoxidase Activity?**

Iron depletion exerts a multitude of effects. The impact of reduced iron levels on vascular function in vivo would be expected to derive from all relevant effects in addition to changes in the concentration of iron within vascular tissue. The finding of Zheng et al that serum nitrotyrosine is lower in frequent blood donors is consistent with the suggestion that lowering iron levels decreases the activity of myeloperoxidase in a clinically significant manner. A significant proportion of serum nitrotyrosine appears to be derived from myeloperoxidase-dependent reactions. The emergence of myeloperoxidase as an important coronary risk factor underscores the links among iron, oxidative stress, and inflammation in cardiovascular disease. Myeloperoxidase contains iron, and its level can be lower in low iron states. Decreased activity of potentially injurious iron-dependent enzymes was proposed as one of a number of mechanisms by which iron depletion might protect against ischemic heart disease. Another example of an iron-dependent enzyme of potential importance is 5-lipoxygenase.

Diminished inflammatory injury in association with reduction in myeloperoxidase activity by induced iron deficiency was, to my knowledge, first demonstrated in 1989. Myeloperoxidase is thus a potentially modifiable coronary risk factor whose level may be decreased by iron removal. It is a target for intervention, not only a disease marker. Sustained iron depletion could be a feasible method for achieving stable, clinically significant myeloperoxidase reductions. Additional studies are needed to define exactly how iron depletion can lower myeloperoxidase activity and how much of a decrease in activity is achievable. Iron may be a limiting factor for myeloperoxidase formation at very low levels. It is also possible that some decrease in activity is an indirect result of a global anti-inflammatory effect of the removal of storage iron. The size of iron status-dependent differences in myeloperoxidase activity may be exaggerated in inflammatory processes. There may be significant complexities in the distribution of activity in diseased tissues, eg, within atherosclerotic lesions. The recent work of Uritski et al on the effects of moderate or severe iron deficiency on myeloperoxidase in an experimental model of colitis illustrates this possibility.

Myeloperoxidase was recently shown to correlate inversely and highly significantly with flow-mediated dilation in human subjects. Flow-mediated dilation in the lowest versus the highest quartiles for serum myeloperoxidase activity were 11.1±6.0% versus 6.4±4.5%. The magnitude of the increase in flow-mediated dilation with frequent donation in the study of Zheng et al is within the same order of magnitude as the increase associated with lower myeloperoxidase activity. The magnitude of these effects suggests that an iron depletion–dependent decrease in myeloperoxidase activity could be sufficient to explain the effect of frequent blood donation.

**Increased Apotransferrin in Frequent Donors**

In iron deficiency, an increase in total iron binding capacity (TIBC) and a decrease in transferrin saturation are seen. These changes produce the well known increase in circulating apotransferrin concentration associated with uncomplicated iron deficiency. The high frequency donation group shows this effect to some extent even though these subjects were not anemic. Free transferrin is a potent antioxidant and also binds free iron. The higher level of apotransferrin in the high frequency donor group could be acting to increase dilation by a mechanism similar to that of exogenous iron chelators.

**Iron Status In Vivo, Vascular Reactivity, and the Iron Hypothesis**

More than 2 decades ago, it was proposed that iron depletion protects against ischemic heart disease and that this effect may explain the remarkably low incidence of cardiovascular events in menstruating women. In the ensuing years of controversy over the validity of the idea, there have been persistent misconceptions on issues of fundamental importance. Zheng et al specifically address some of the key sources of confusion in the debate on the iron hypothesis. Iron depletion is often misconstrued to mean “a process of iron loss,” rather than the particular condition of having essentially no iron in storage but with a hemoglobin value in the normal range.

This misreading of the hypothesis can result in poor study design in work on blood donation and heart disease. Casual blood donation is a process that involves iron loss, but it may have little impact on cardiovascular risk if not sufficiently regular and frequent to produce sustained iron depletion. Previous work on the effect of blood donation on cardiovascular disease has been inconsistent, with 3 studies supporting a protective effect and 1 negative report. The only study using a design similar to that of Zheng et al, ie, a comparison of frequent with infrequent donors, found lower cardiovascular disease rates in frequent donors. A design involving only donors is inherently stronger than one comparing donors with non-donors because of a lower possibility of selection bias.

Future studies following up Zheng et al should include a prospective randomized trial of the effects of iron depletion or near iron deficiency on vascular reactivity. It is important to establish what maximum increase in flow-mediated dilation might be achievable with iron reduction therapy. The findings of Zheng et al do not rule out even greater increases...
in flow-mediated dilation in actual iron deficiency. Future studies should include direct measurement of myeloperoxidase activities and the effect of iron reduction on its level. These determinations could be helpful in assessing the relative contribution of lower myeloperoxidase activity versus change in vascular iron status to improved endothelial function. Additional epidemiological correlates that should be specifically sought in future work include the roles of stored iron acquisition as an explanation for decreased flow-mediated dilation with age and with the menopausal transition.

**Concluding Comment**

A reasonable interpretation of the findings of Zheng et al is that reduction of stored iron level from a conventionally low level to a conventionally very low level is the factor responsible for the significant increase in flow-mediated dilation. Such an interpretation is consistent with a body of in vitro data on iron and endothelial cell function, with earlier work showing that iron chelation improves endothelial function clinically, with the improvement in flow-mediated dilation after phlebotomy treatment in rheumatoid arthritis patients, and with the previously stated hypothesis on the role of iron in ischemic heart disease. However, there may be a particular impetus to interpretive caution in explaining the work of Zheng et al because of the persistent and widespread belief that stored iron at a conventionally normal level is entirely benign. The results of Zheng et al suggest that an iron-depleted subject experiences a significant decrease in flow-mediated dilation with the acquisition of essentially any stored iron. A definitive intervention study to verify that reduction of stored iron level from a conventionally low level to a conventionally very low level is the factor responsible for the significant increase in flow-mediated dilation with age and with the menopausal transition. Such a study should give valuable insight into endothelial function in vivo and could provide a new basis for evaluating the fundamental safety of storage iron.

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