

## Measures of vascular reactivity: prognostic crystal ball or Pandora's box?

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THE VESSEL WALL is the final common pathway for the impact of cardiovascular risk factors and genetic predisposition to atherothrombotic events, and its health and function may therefore reflect aggregate cardiovascular risk. The quest to find a noninvasive prognostic tool to assess vasculature health has led to the development of a number of different measurement approaches. The rationale for finding a prognostic tool based on assessment of vascular reactivity is clear: major cardiovascular risk factors that contribute to the Framingham score [age, total cholesterol, high-density lipoprotein (HDL) cholesterol, systolic blood pressure, treatment for hypertension, and cigarette smoking] fail to explain ~50% of cardiovascular morbidity and mortality (11). Developing a tool to aid in the prediction of micro- and macrovascular disease would therefore greatly improve the odds of identifying those at highest risk and might also provide a basis for treat-to-target approaches for interventions such as exercise training.

The search for a simple, noninvasive, and widely applicable test that can be applied routinely in a clinical setting is not straightforward. There is currently much confusion among investigators and clinicians regarding which tests measure which aspect of vascular function, and whether any one test can predict subsequent cardiovascular risk. Do all measures of vascular reactivity provide similar information about health of the vascular system or atherosclerotic risk? Does an approach that measures macrovascular function tell us the same information as a test of microvascular function? Are changes in vascular health in both vessel types manifest over the same time course?

In a study in the *Journal of Applied Physiology*, Dhindsa and colleagues (6) compared seven different methods used to evaluate vascular reactivity in humans using noninvasive approaches. The goal of the study was to assess interrelationships among different noninvasive measures that have been used to measure micro- and macrovascular reactivity. The authors included two measures of conduit artery function [flow-mediated dilation (FMD) and pulse wave velocity (PWV)] and four measures of resistance or microvascular function [forearm reactive hyperemia; reactive hyperemia index by finger plethysmography (RHI); skin reactive hyperemia; and fingertip temperature rebound]. Unique aspects of the study include that measurements were all performed simultaneously, allowing the authors to make direct comparisons of the various approaches, and that all tests utilized the postischemic vascular response to forearm occlusion.

Of the measures of vascular reactivity compared, FMD is the most widely used and has received the most scrutiny and evaluation. Recently, FMD has been demonstrated to provide

independent prognostic value when added to traditional measures of cardiovascular risk in postmenopausal women (11). In several studies FMD was significantly correlated with coronary artery function (1, 13), and a large and accumulating body of evidence has suggested that both peripheral and coronary FMD are strongly correlated and independently prognostic by multivariate analysis that includes traditional risk factors (5). This is not to say, however, that FMD testing is either simple or ready to be applied as a clinical diagnostic tool. Dhindsa and colleagues (6) highlight that FMD testing requires expensive equipment and highly trained personnel. Furthermore, despite efforts to standardize FMD testing (4), there remains variability in the way the procedure is performed and particularly in the way the resulting data are analyzed. For example, some investigators standardize the time at which diameter is measured following release of occlusion (e.g., at 60 s), in theory to obtain maximal artery diameter. However, it has recently been demonstrated that there is wide variability in time to peak diameter across subjects, and particularly across subject groups, such that the interpretation of findings can differ according to which approaches are employed (2). In addition, shear stress stimulus quantification remains steadfastly ignored in the clinical literature, despite the strong likelihood that some reported differences between groups and within subjects following interventions may simply reflect differences in the dilator stimulus.

Notwithstanding these issues, our knowledge of the mechanisms behind FMD testing is more complete than the other measures evaluated by Dhindsa and colleagues. FMD has been demonstrated to be almost entirely mediated by NO when appropriate techniques are used, and NO has been identified as an important antiatherogenic molecule (3). FMD is therefore a good overall indicator of endothelial function, vascular health, and likely atherosclerotic risk, particularly in research studies. However, it can also be argued that a simpler test of vascular reactivity would be optimal if the concept of direct vascular health assessment is to make the clinical “primetime.”

Perhaps not surprisingly, Dhindsa and colleagues (6) found little association between the measurements of microvascular versus macrovascular function, with FMD being only modestly associated with RHI and finger temperature rebound. To further compare these modest associations, Dhindsa and colleagues stratified subjects into tertiles and quartiles based on their brachial artery FMD response. However, statistical significance was not achieved with this approach, despite studying 40 subjects. Interestingly, when brachial FMD was previously compared with the “gold standard” of endothelial function testing in resistance vessels (i.e. intra-arterial infusion of ACh into the brachial artery), the two methods were not significantly related (7). Changes in conduit and resistance vessel function with exercise training are also only modestly correlated (8). As pointed out by Dhindsa and colleagues (6), the present findings provide further evidence that the mechanisms involved in vascular reac-

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tivity of the macro- and microvasculature are quite different. This is reinforced by a comparison of correlations between peripheral and coronary vascular function: peripheral FMD is quite modestly correlated with coronary ACh responses (1), whereas peripheral and coronary FMD (13) and peripheral and coronary ACh responses (12) are strongly associated. Of course, comparisons of like-for-like mechanisms produce much stronger correlations than comparisons undertaken between responses at different levels of the arterial tree, where mechanisms responsible for functional adaptations are known to differ. The latter point is reinforced by the lack of a strong association between FMD and PWV in the present study, ostensibly both indexes of macrovascular function, which nonetheless assess different aspects of vascular reactivity.

It is important to point out that vascular reactivity may be affected by many different factors, of which endothelial function is only one aspect. We would be remiss not to point out that Dhindsa and colleagues only recruited healthy individuals, which may raise concerns that the predictive ability of the tests for a clinical condition were not being compared. However, there was a wide age range of subjects (19–65 yr), and, importantly, the subject population studied resulted in a wide range of FMD responses. The authors sought to minimize the influence of chronic diseases and risk factors in an effort to study the associations of different methodologies in more physiological, rather than pathological, states.

Most surprising in this study was the relative lack of association found between all the measures, considering they were made at the same time, in the same subjects, to the same stimulus. In fact, of the 21 possible one-to-one comparisons, only 5 associations reached statistical significance. Of these, the highest correlation coefficient was 0.55 between two measures of microvascular function: skin reactive hyperemia and RHI in the fingertip with pulse amplitude tonometry, two tests that are likely heavily influenced by the cutaneous circulation. Due to its accessibility, the cutaneous circulation seems a logical choice to measure vascular reactivity or endothelial function if the goal is to find a simple and noninvasive approach. The suggestion has been recently made that the skin can be used as a surrogate measure of vascular reactivity or as an index of global microvascular function (9). However, well-established regional differences exist in skin vascular function, and the physiology of the skin and pulp at the tip of the finger, which serves as the site of measurement for RHI, is primarily composed of arteriovenous anastomoses, which possess unique physiological and structural characteristics. About 60% of the RHI response is mediated by NO, whereas cutaneous reactive hyperemia has been shown to be dependent on an endothelium-derived hyperpolarizing factor (EDHF) pathway (10), rather than NO. Thus an association between these two measures is difficult to interpret in a physiological or mechanistic context.

The paper by Dhindsa and colleagues (6) therefore raises important concerns about the current raft of measurements of vascular reactivity, and particularly about our ability to make comparisons across studies in which different methods of assessment are employed. Many of the approaches have not been sufficiently evaluated to know whether they have the potential to add prognostic value to cardiovascular risk assessment. The experience of the last decade of explosion in FMD-related research, where clinical enthusiasm preceded careful assessment of the underlying mechanisms, provides a cautionary note and a reason for emphasis on careful preliminary physiological

research before adoption of yet more indirect measures of vascular function or health. It is also conceivable, indeed likely, that the optimal surrogate indexes of vascular health and risk will differ at distinct levels of the arterial tree and that such indexes will provide independent, but perhaps complementary, prognostic information.

In summary, a good measure of vascular reactivity aimed at improving cardiovascular risk assessment should possess the following characteristics: 1) it should have a sound physiological and mechanistic basis that links it to atherothrombotic risk; 2) it should independently add to the prediction of cardiovascular events, above and beyond established risk factor measurements; 3) it should be reproducible, observer independent, and easily standardized; and 4) an improvement in the test should predict an improvement of subsequent cardiovascular risk.

Although the goal to develop a simple, noninvasive test of vascular reactivity is laudable, much work remains to bring a “crystal ball” into clinical practice that will provide real prognostic value in cardiovascular risk assessment. This will only be accomplished by thoughtful examination of the proposed test, the underlying physiological mechanisms engaged, and the demonstrated ability to predict morbidity and mortality.

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