Lipocalins and Insulin Resistance: Etiological Role of Retinol-Binding Protein 4 and Lipocalin-2?

The prevalence of type 2 diabetes is increasing dramatically worldwide. Excess adiposity is an important contributor to the development of type 2 diabetes and cardiovascular diseases (1). Insulin resistance, inflammation, hypertension, and dyslipidemia, components of the metabolic syndrome, have been implicated in the effects of adiposity on type 2 diabetes and cardiovascular diseases, but the mechanisms responsible for these detrimental effects of adiposity have not been fully elucidated.

Two paradigms are currently areas of intense study: one focused on ectopic fat and the other on the endocrine function of adipose tissue (2). Ectopic fat is present in nonadipose tissues such as the liver, muscle, and probably pancreatic β-cells. Both lipodystrophy (failure to develop adipose tissue) and obesity with full adipose cells are characterized by a lack of fat-storage capacity, resulting in overflow to other tissues of triglycerides and free fatty acids in the form of ectopic fat. Normal physiological processes can be disrupted by this ectopic fat, leading to insulin resistance and impaired insulin secretion. The endocrine function of adipose tissue is an important regulatory process throughout the body that is carried out by signaling proteins secreted by adipose tissue. These signaling proteins are called adipocytokines or adipokines, and they include leptin, adiponectin, resistin, tumor necrosis factor-α, and interleukin-6.

Members of the lipocalin family of proteins have large sequence differences but share a common tertiary structure formed by segments termed lipocalin folds (3). Lipocalin folds consist of 8 antiparallel β-sheets that surround a hydrophobic pocket and allow lipocalins to function as transport or carrier proteins. Several human lipocalins have been identified, including retinol-binding protein 4 (RBP4), which has recently been added to the list of adipokines that may link obesity and insulin resistance (4, 5). In this issue of Clinical Chemistry, Dr. Wang and colleagues report on the association of lipocalin-2 with obesity, insulin resistance, and inflammation in mice and humans (6). Lipocalin-2 (also known as neutrophil gelatinase-associated lipocalin, siderocalin, 24p3, and uterocalin) has previously been shown to play a role in the innate immune response to infection by binding to iron-laden bacterial siderophores and thereby limiting bacterial growth (7). Wang et al. now report that circulating concentrations of lipocalin-2, as well as expression of lipocalin-2 in adipose and liver tissue, were increased in db/db obese diabetic mice compared with normal mice (6). Furthermore, plasma lipocalin-2 concentrations were higher in obese than in lean humans and were correlated with body mass index (BMI) and various components of the metabolic syndrome. After adjustment for BMI, statistically significant associations remained with fasting glucose, the Homeostasis Model Assessment (HOMA) index for insulin resistance, and C-reactive protein concentration. Treatment with the peroxisome proliferator–activated receptor-γ agonist rosiglitazone markedly decreased lipocalin-2 expression in mice and circulating concentrations in both mice and humans. These changes in lipocalin-2 were correlated with changes in C-reactive protein and HOMA insulin resistance (HOMA-IR) index. The authors should be commended for conducting this careful study using various techniques in both mice and humans. Their intriguing results raise the question whether lipocalin-2 can be useful as a predictor of cardiovascular diseases and whether lipocalin-2 has causal effects on insulin resistance, hyperglycemia, or inflammation and could thus be a treatment target.

In the human study reported by Wang et al., the association between lipocalin-2 concentrations and indicators of glucose metabolism was attenuated after adjustment for BMI: No significant association remained with type 2 diabetes, 2-h plasma glucose concentrations, or fasting insulin concentrations, and only modest correlations remained with fasting glucose and the HOMA-IR index (6). Given that BMI may not have fully captured the effect of body fat or specific body fat depots, the strength of the reported associations independent of body fatness may have been overestimated. However, the parallel decrease in lipocalin-2 and HOMA-IR after rosiglitazone treatment provides some reassurance that cross-sectional associations with insulin resistance are not solely attributable to incomplete control for body fatness. Possibly, the independent associations with HOMA-IR and fasting glucose, but not 2-h glucose concentrations, reflect a specific association between lipocalin-2 and hepatic insulin sensitivity or glucose output rather than peripheral insulin sensitivity (8). Further human studies with more precise measures of body fatness and glucose homeostasis are needed to confirm the associations reported by Wang et al. (6).

Decreased adipocyte expression of glucose transporter 4 (GLUT4) is associated with systemic insulin resistance, and based on studies in mice, it has been postulated that RBP4 acts as the mechanistic link by which decreased adipocyte GLUT4 expression contributes to insulin resistance (4). Circulating RBP4 concentrations were increased in insulin-resistant mice. Moreover, transgenic overexpression of human RBP4 and injection of recombinant RBP4 decreased insulin sensitivity in normal mice, whereas genetic deletion of the RBP4 (retinol binding protein 4, plasma) gene or normalization of RBP4 concentrations in obese mice improved insulin sensitivity. RBP4 concentrations were also increased in obese humans, and higher concentrations were correlated with lower insulin.
sensitivity and other components of the metabolic syndrome (4, 5). Results in humans have not been entirely consistent, however, and lack of associations with type 2 diabetes, obesity, and fasting insulin levels have also been reported (9–11). An inverse association between adipocyte GLUT4 protein and serum RBP4 concentrations in humans has been reported (5), but other researchers reported a positive association between adipocyte GLUT4 expression and RBP4 (11). Despite impressive results obtained in animal studies, findings in humans have been inconsistent, and further studies are clearly warranted.

Proving causal effects of a biomarker on diseases in humans is exceedingly difficult, but several methodological approaches can be useful. Given that temporality (i.e., a cause preceding an effect) is a prerequisite for causality, prospective studies that assess the biomarker before occurrence of disease are critical. Even if increased biomarker concentrations in apparently healthy people predict the disease risk, however, these increased concentrations may still reflect preclinical disease or confounding by a third factor that affects both the biomarker of interest and disease risk. For example, if increased lipocalin-2 concentrations were to be associated with an increased incidence of type 2 diabetes, this association could still be confounded by other inflammatory processes. Another approach, the study of genetic variants that substantially and selectively affect concentrations of the biomarker in relation to disease incidence, can provide evidence for causality or lack thereof. This approach has been termed Mendelian randomization to reflect the random assortment of genes from parents to offspring and, as a result, the lack of confounding and reverse causation expected in studies of these variants (12). This approach requires the assumption that there is no linkage disequilibrium of the genetic variant of interest with a nearby variant that affects related biological processes. Researchers have attempted to prove the lack of causal effect of fibrinogen on the development of coronary heart disease using this method, but findings may have been affected by measurement error and other biases (12). A third approach, randomized controlled trials that administer substances that selectively inhibit action of the biomarker, could provide evidence for a causal effect. Agents such as proliferator-activated receptor-γ agonists that have pleiotropic effects are less useful for such investigations, whereas studies of the effect of fenretinide or related compounds that selectively decrease RBP4 are promising for elucidating the effects of this lipocalin on insulin resistance in humans (4). The possibility must be considered, however, that fenretinide has effects independent of its action on RBP4, and it is unclear whether similar agents would be useful for the study of selective lipocalin-2 decrease in humans. Wang et al. mention that they have unpublished observations suggesting that administration of a neutralizing antibody that blocks the action of lipocalin-2 can alleviate insulin resistance in mice (6).

Irrespective of the question of whether associations reflect causal effects, lipocalin-2 or RBP4 could be used as markers for the prediction of cardiovascular diseases. The use of various novel biomarkers, including lipoprotein-associated phospholipase A2 and C-reactive protein, in addition to traditional risk factors has been suggested to enhance the prediction of cardiovascular diseases and may be useful for the identification of high-risk persons needing treatment (13). Prospective evaluation is needed to assess the added predictive value of lipocalin-2 concentrations beyond more established markers of cardiovascular risk. In addition to consistent and independent findings from diverse populations, the availability of standardized, inexpensive assays is also required before use in clinical practice can be considered.

The study of RBP4, lipocalin-2, and other adipokines can contribute to our understanding of the etiology of insulin resistance and related metabolic disorders and may ultimately lead to the discovery of novel therapies for these conditions. The promise of ongoing research should not deter us, however, from optimizing available strategies for preventing and treating obesity and related morbidity. In particular, increased physical activity, a prudent diet, and modest weight loss through changes in energy balance are known to improve insulin sensitivity and substantially decrease risk of type 2 diabetes and cardiovascular diseases (14, 15).

References


Rob M. van Dam*
Frank B. Hu

Departments of Nutrition and Epidemiology
Harvard School of Public Health
Boston MA

Channing Laboratory
Brigham and Women’s Hospital
and Harvard Medical School
Boston, MA