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Bilirubin: A Potential Biomarker and Therapeutic Target for Diabetic Nephropathy

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Diabetic nephropathy (DN), which is a major end-organ complication in diabetes, continues to be the most common cause of end-stage renal disease and accounts for >40% of patients on renal replacement therapy (1). Currently available factors that are routinely used in clinical practice to predict and monitor the progression of DN include degree of proteinuria and both glycemic and blood pressure control. In this issue of *Diabetes*, Riphagen et al. (2) performed a post hoc analysis of two large clinical trials—Reduction of Endpoints in NIDDM With the Angiotensin II Antagonist Losartan (RENAAL) and Irbesartan Diabetic Nephropathy Trial (IDNT)—and demonstrated that serum bilirubin levels are inversely correlated with progression of DN. These findings are clinically important because they identify a potential biomarker and/or therapeutic target for DN, a disease that causes significant morbidity and mortality.

Bilirubin is generated when heme oxygenase (HO) catalyzes the degradation of heme (derived from heme proteins). This results in formation of biliverdin, which is rapidly converted into bilirubin by biliverdin reductase (Fig. 1) (3). Further processing of bilirubin occurs in hepatocytes, where unconjugated (lipid-soluble) bilirubin is conjugated by uridine diphosphate–glucuronosyl transferase (UDP-GT) to a water-soluble form for excretion. Total bilirubin is the sum of conjugated (direct) and unconjugated (indirect) bilirubin and generally ranges from 0.3 to 1.2 mg/dL in healthy individuals. In conditions such as erythroblastosis fetalis, hemolysis results in markedly elevated bilirubin, which causes kernicterus and neurological damage in neonates. Furthermore, genetic deficiency of UDP-GT in Crigler-Najjar syndrome type I results in severely elevated total bilirubin and is incompatible with life. Given the outcomes of these conditions, it is not surprising that bilirubin was long considered to be merely a toxic byproduct of heme degradation. However, unconjugated bilirubin levels are positively correlated with plasma antioxidant capacity (4), and moderate elevations in serum total bilirubin have been associated with reduced susceptibility to several common diseases (Table 1). Although a protective role for bilirubin in kidney disease has been established in animal studies (5,6), human data are lacking in this area.

Riphagen et al. (2) investigated the association between serum bilirubin levels and progression of DN in a post hoc analysis of 1,498 patients in the RENAAL trial (7). Data from this study were then independently verified using the IDNT (8). In both trials, the renal end point was examined over a ≥2.5-year follow-up period and defined as a doubling of serum creatinine or end-stage renal disease requiring dialysis or transplantation. The authors' findings demonstrate, for the first time, a significant and graded inverse association between baseline total bilirubin levels and progression of DN in patients between 30 and 70 years of age. This association remained significant after adjustment for potentially confounding patient characteristics and risk factors for renal disease, and it was independent of treatment with placebo or angiotensin receptor blocker. While serum total bilirubin levels decreased significantly from baseline during the first year of the study, this change was not associated with progression of DN and may be explained by the observed concomitant decrease in serum hemoglobin levels, an observation that was exacerbated by angiotensin receptor blocker therapy (losartan, RENAAL; irbesartan, IDNT) relative to control (2). These findings suggest that measurement of bilirubin levels may identify subjects at risk for progression of DN.

Confirmation of initial findings using the IDNT and the relatively large sample size strengthens the findings

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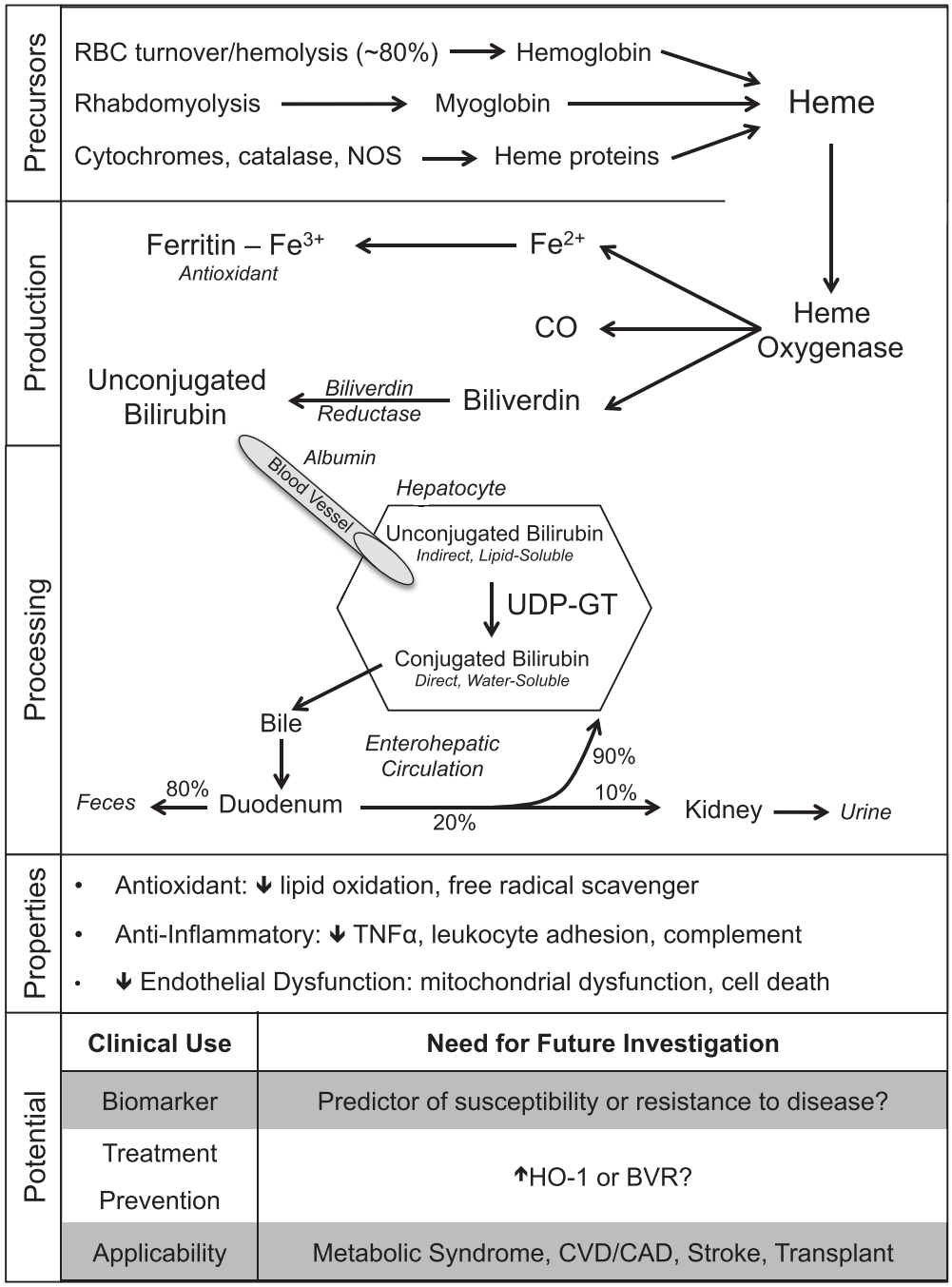

Figure 1—Schematic of bilirubin generation and conjugation in the liver. The physiological properties of bilirubin may explain its ability to protect against the progression of DN. These properties make bilirubin a potential clinical biomarker or therapeutic target for a variety of disease states. BVR, biliverdin reductase; CAD, coronary artery disease; CO, carbon monoxide; CVD, cardiovascular disease; Fe^{2+}, ferrous iron; Fe^{3+}, ferric iron; NOS, nitric oxide synthase; RBC, erythrocyte; TNFα, tumor necrosis factor-α.

reported by Riphagen et al. However, several limitations inherent to the design of this study deserve mention. Given that the authors were constrained to the data gathered in the RENAAL trial and the IDNT, only total bilirubin levels in a narrow range (<1.5 times the upper limit of normal) were studied. To translate these findings to clinical practice, future studies are needed to define an optimal range of total bilirubin in which progression of DN is prevented. In addition, only total bilirubin levels were measured, without distinguishing between conjugated versus unconjugated bilirubin. This is particularly important since conjugation of bilirubin appears to significantly affect its functional characteristics. Moderate unconjugated hyperbilirubinemia (ranging between 1.2 and 6.0 mg/dL) that is seen in patients with Gilbert syndrome confers protection from cardiovascular disease (9). Such

Table 1—Clinical conditions associated with serum bilirubin levels

Condition	Strength of evidence
Cardiovascular disease	
Atherosclerosis	+++
In-stent restenosis	+
Cerebral vascular disease (stroke)	++
Metabolic syndrome	++
Diabetes complications	
DN	++
Retinopathy	+
Chronic kidney disease	+
Transplant rejection	+
Autoimmunity	
Systemic lupus erythematosus	+
Rheumatoid arthritis	+

+++, Numerous prospective and retrospective clinical studies, large patient base, animal studies yielding some degree of mechanistic insight. ++, Fewer human studies, less mechanistic insight from animal data. +, Observational studies, limited by sample size (15,25–27).

a protective effect has not been reported in patients with conjugated hyperbilirubinemia caused by obstructive jaundice or genetic deficiencies such as Dubin-Johnson syndrome (4,10). An additional limitation of this study is that its design limits the ability to establish a causal link between serum total bilirubin and protection from progression of DN.

Nonetheless, this report raises questions about how bilirubin is generated and why its concentration in serum varies between individuals. The HO enzyme system, comprised of two isoforms, HO-1 and HO-2, degrades the noxious pro-oxidant heme moiety, resulting in the production of biliverdin, carbon monoxide, and iron (11). Biliverdin is converted to bilirubin by biliverdin reductase, an enzyme that has key roles in cell signaling via the protein kinase C pathway (12). It has been suggested that individuals with higher HO-1 expression also have increased total bilirubin (13). Therefore, whether elevated serum bilirubin directly provides protection from disease or is simply a marker of increased HO-1 expression remains to be verified experimentally. While HO-2 is expressed constitutively, HO-1 expression is induced by numerous stimuli that impose cellular oxidative stress. Genetic polymorphisms in the human HO-1 proximal promoter—specifically, length variations in a GT repeat region—affect basal and inducible HO-1 expression and correlate with several diseases, including chronic kidney disease (14). Animal models have demonstrated that increased HO-1 expression confers protection in many diseases. Several ongoing clinical trials targeting the HO-1 pathway are focused on diseases where an association between increased serum bilirubin levels and protection from disease was demonstrated (15,16) (Table 1). It is important to underscore that similar to the underlying vasculopathy in diabetes complications, many diseases that are associated with bilirubin levels are also mediated by vascular injury (Table 1). This commonality among diverse pathologies supports a protective role for bilirubin at the level of the vasculature—the endothelium and/or vascular smooth muscle.

In diabetes, the protective properties of bilirubin are likely due to its potent antioxidant properties (Fig. 1). Bilirubin inhibits lipid peroxidation and attenuates LDL oxidation (17). Chronic hyperglycemia results in the generation of reactive oxygen species (e.g., superoxide), predominantly by the mitochondria. This pathway is particularly prominent in endothelial cells and leads to vascular dysfunction, a major underlying feature in both diabetes and its complications (18). Targeting mitochondrial oxidative stress prevents DN in mice (19). In addition, bilirubin derived from HO enzyme activity or administered exogenously prevents endothelial cell death in diabetic rats (20). Exogenous bilirubin also preserves mitochondrial integrity (5). Aortic rings isolated from hyperbilirubinemic Gunn rats, which have a genetic deficiency in UDP-GT, exhibit reduced levels of superoxide production and a blunted tonic response to angiotensin II infusion (21). These findings were recently corroborated in humans with Gilbert syndrome, where hyperbilirubinemia was associated with decreased indices of oxidative stress and enhanced endothelium-dependent vasodilation (22). Further, the protective properties of bilirubin in vascular disease have been confirmed in other models of systemic hypertension (23) as well as a model of balloon injury (24).

Given the emerging understanding of the protective properties of bilirubin and its broad association with disease in clinical trials, the results published in this issue of *Diabetes* have the potential to positively impact the management of patients with diabetes and its complications. Importantly, bilirubin measurement is inexpensive, performed routinely, and accessible to most medical centers. Therefore, the findings reported by Riphagen et al. (2) are widely applicable, especially given the staggering burden of DN. This study also demonstrates that serum bilirubin levels can be used by clinicians to prognosticate the progression of DN. However, additional work must be done to understand the full clinical potential of bilirubin as a marker in monitoring DN (Fig. 1). In the future, studies should be designed to determine the reliability of bilirubin in estimating the rate of DN progression for the purpose of treatment planning. Animal models of hyperbilirubinemia such as the Gunn rat in the context of diabetic kidney disease may be an important area for investigation. Future research should focus on clearly defining how (prognostic versus therapeutic), when (specific disease states to which bilirubin is causally linked), and why (mechanism) bilirubin may be

a valuable clinical tool in assessing and managing the progression of DN.

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