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Haptoglobin Genotype and Its Role in Diabetic Cardiovascular Disease

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Abstract

Over the past decade, several longitudinal epidemiological studies have brought attention to the haptoglobin genotype and its importance in determining diabetic vascular disease risk. This manuscript presents an overview of the biology of the haptoglobin genotype and reviews the literature concerning its role in the development of cardiovascular disease among individuals with diabetes mellitus.

Keywords

Haptoglobin genotype; Cardiovascular disease; Diabetes

Introduction

Despite advances in the health care of patients with diabetes as well as in prevention efforts for the development and treatment of diabetes complications, the life expectancy for such patients remains suboptimal compared to the general population [1, 2]. This vast discrepancy is largely attributed to a greater cardiovascular disease risk associated with this metabolic disorder, which comprises the leading cause of death in this population [1-4]. Thus, although rates of cardiovascular disease have declined over time in diabetes [5], US national data suggest that reductions in all-cause and cardiovascular mortality have occurred only among men [6]. In women, all-cause mortality has actually increased and cardiovascular disease mortality rates have remained rather stagnant [6]. Additionally, no reductions have been observed in either cardiovascular disease events or mortality in the subgroup of individuals with type 1 diabetes [7].

It is becoming apparent that the noted [8] improvements in traditional risk factor management as well as lifestyle modifications may not present a sufficient cardiovascular disease prevention strategy for this high-risk subgroup, the size of which continues to rise worldwide and which, at present, constitutes over 8 % of the US population [4]. Clearly, aggressive management of traditional risk factors should persist, perhaps even with greater vigor among women. The realization, however, that their combined contribution may not

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Potential Conflict of Interest Dr. Levy is the author of a patent which is owned by his university which claims that the Hp genotype can predict the development of diabetic vascular complications. This patent has been licensed by Haptocure. Dr. Levy is serving as a consultant for Haptocure.

account for all the excess cardiovascular risk in diabetes demonstrates a need to identify factors that explain a further large residual part of this excess risk. The observation of resistance to vascular diabetic complication development despite poor glycemic control [9] or lack of risk reduction given improved blood glucose levels in a large subgroup of individuals with diabetes [10-12] suggests that genetic susceptibility may play an important role.

Indeed, researchers have long suggested an influence of genes in the development of both microvascular and macrovascular complications of diabetes [13, 14]. However, despite extensive data on familial aggregation of diabetic nephropathy, similar studies on cardiovascular disease were lacking until the development of technologies assessing subclinical atherosclerosis, i.e., intimal medial thickness and coronary artery calcification [15-18]. To date, familial aggregation of medial artery calcification has been shown among Pima Indians with diabetes [19] as well as in the Diabetes Heart Study among families having multiple members with type 2 diabetes [20]. Investigators of the latter study have further provided evidence that coronary artery calcification also has a significant genetic component [21].

Nevertheless, identifying genes that contribute to complication development has been challenging, given the power requirements in tandem with the sample size constraints of even the largest existing cohorts, especially in the case of type 1 diabetes, and the vast number of potential candidate genes to investigate. Caution should further be exercised in interpreting findings resulting from genome-wide associations, especially in cases where polymorphism functionality and/or biologic rationale have not been previously established. Nonetheless, the predictive ability of known functional polymorphisms for vascular diabetes complications has been previously assessed in a number of studies [13, 14]. Over the past decade, evidence has been accumulating that one such functional polymorphism in the haptoglobin (Hp) gene is an independent risk factor for the development of vascular disease risk among individuals with diabetes, both type 1 and type 2 diabetes. Hp and its importance in diabetic cardiovascular disease risk is, therefore, the focus of the present review. In this manuscript, we have sought to provide an overview of the biology of the Hp genotype as well as evidence from in vitro, animal and human studies relating it to the development of cardiovascular complications of diabetes.

Haptoglobin: Discovery, Physiology, Structure, and Function

Polonovski and Jayle [22] were the first to describe the presence in serum of a hemoglobin-binding protein in 1938. They named this protein, which they later characterized as an alpha$_2$-glycoprotein, haptoglobin (Hp), from the Greek word *haptein* (to bind) and (hemo)globin, after its hemoglobin-binding properties [23]. It is now known that the biosynthesis of Hp occurs predominantly by liver hepatocytes, although the Hp gene is also expressed in other cell types and in several other organs, such as the lung, the kidney, the spleen, and the heart [24-26]. The serum concentration of Hp becomes measurable by the first month of life and reaches adult levels by 6 months of age, ranging, in a healthy state, from 30 to 300 mg/dL [27]. The intra-individual concentration of Hp is fairly small; however, being an acute-phase protein, the serum levels of Hp can increase dramatically in response to injury, infection, or inflammation [28].

Hp has been attributed diverse biological functions. Its major role, however, is to bind free hemoglobin released daily from intravascular hemolysis in an essentially irreversible, non-covalent association (K_d~10^{-15}) [29]. In normal physiology, the concentration of Hp in plasma is far in excess of free hemoglobin [30], and thus, all hemoglobin released from intravascular hemolysis is rapidly bound to Hp. The formed Hp–hemoglobin complex is

then cleared from circulation by the monocyte/macrophage CD163 receptor. Macrophage-like Kupfer cells are the predominate site of clearance of the Hp–hemoglobin complex [31]. Indeed, although the half-life of Hp is approximately 3–5 days, when bound to hemoglobin, the complex is removed from the circulation within 20 min [32]. This hemoglobin-binding property of Hp is crucial to normal physiology for a number of reasons. The first is that free hemoglobin is highly toxic and has the ability to induce considerable oxidative tissue damage through the release of its heme iron [33]. Hp, by binding to free hemoglobin, inhibits heme iron release and thus reduces free hemoglobin's oxidative potential [29]. Importantly, this mechanism serves to reduce glomerular filtration and, thus, loss of both hemoglobin and iron following erythrocyte destruction as well as reduce kidney damage, resulting from increased iron deposition in renal proximal tubule cells [30, 34]. An additional reason is that hemoglobin is a potent scavenger of nitric oxide, a central modulator of vascular tone and function [35]. Thus, although hemoglobin scavenges nitric oxide at the same rate regardless of whether it is bound or unbound to Hp, differences in the rate of clearance of the Hp–hemoglobin complex according to Hp genotype may affect nitric oxide bioavailability [36]. As both oxidative injury and diminished availability of nitric oxide are thought to contribute to the development of vascular complications in diabetes [37-39], it is evident that Hp has the potential to be a determinant of diabetic vascular disease risk.

The Haptoglobin Polymorphism

In 1955, Smithies [40] identified the presence of a genetic polymorphism in the Hp gene. The Hp class 1 allele has five exons and is remarkably conserved between all species. The Hp class 2 allele, present only in humans, appears to have arisen from the Hp 1 allele early in human evolution by a duplication of exons 3 and 4 of the Hp 1 allele [30]. As a result of the duplication of the portion of the Hp gene involved in Hp monomer multimerization, the stoichiometry of the mature Hp protein is Hp genotype-dependent. In individuals homozygous for the Hp 1 allele (Hp 1-1), the Hp protein is found only as a dimer, while in individuals homozygous for the Hp 2 allele (Hp 2-2), the Hp protein is found as a cyclic polymer comprising up to ten Hp monomers [41]. The prevalence of the three Hp genotypes varies dramatically across populations, with the highest frequency of the Hp 1 allele found in Africa and South America and the lowest in Southeast Asia. Detailed reviews on the geographic distribution of this genotype have previously been published [30, 42]; in most Western countries, however, the distribution of Hp is 16 % Hp 1-1, 48 % Hp 2-1, and 36 % Hp 2-2 [30].

Several functional differences have been identified between the Hp 1 and Hp 2 protein products. First, although no differences appear to exist in the affinity of the Hp 1 and Hp 2 protein products for hemoglobin [43]—as the α^1-chains are smaller than the α^2-chains—1 g of the Hp 1-1 protein contains more $\alpha\beta$-subunits than 1 g of Hp 2-1 or Hp 2-2 protein, giving the Hp 1-1 protein the ability to bind more hemoglobin than the other two Hp types [30]. Second, due to the smaller size of the Hp 1-1 protein, it is more effective in sieving into environments where the Hp 2-2 protein might be restricted, thereby offering superior protection against hemoglobin in these environments [30]. Third, the Hp 1 protein is more efficient in preventing oxidation by hemoglobin and in preventing heme release from the Hp–hemoglobin complex [43, 44]. Fourth, the Hp 1 allele is more efficient in promoting the uptake of the Hp–hemoglobin complex by the CD163 receptor [43, 45, 46]. Indeed, it has previously been shown that the Hp–hemoglobin complex is cleared from circulation twice as fast in the case of the Hp 1 compared to the Hp 2 protein [47].

Hp is an HDL-associated protein, with the binding site mapped to helix 6 on apolipoprotein A1 [48-50]. Through this binding, Hp has the ability to tether hemoglobin to HDL,

potentially rendering it pro-atherogenic and proinflammatory [51, 52]. This binding to HDL is increased for the Hp 2–hemoglobin complex as its clearance is impaired, resulting in an increased concentration in plasma. However, the Hp 2-2–hemoglobin complex is also redox-active [45]; thus, once bound to HDL, it has the ability to mediate the structural and functional modification of the lipoprotein, inhibiting HDL's ability to promote reverse cholesterol transport and thus rendering it dysfunctional [47]. Such modifications do not occur when the Hp 1-1–hemoglobin complex, which is relatively redox-inert [45], binds to HDL [47].

Relevance of the Haptoglobin Genotype to Diabetes Mellitus

Although the distribution of Hp alleles does not differ between persons with and without diabetes [53], the Hp genotype has been shown to be predictive of vascular outcomes only in those with diabetes. While functional differences in the antioxidant capacity and the clearance of hemoglobin are exaggerated in the setting of diabetes, a compelling reason for this interaction between diabetes and the Hp genotype on vascular outcomes remains enigmatic. The observation of elevated hemoglobin levels in type 1 diabetes relative to the general population [54] may help explain this finding as it would presumably lead to enhanced hemoglobin-based oxidative potential. However, other aspects of the diabetic state may also foster this apparent interaction between the Hp type and diabetes on vascular risk and include the glycosylation of the hemoglobin molecule and the reduction in the proportion of monocytes expressing surface CD163, the latter likely explaining the observation of a greatly increased half-life of the Hp–hemoglobin complex in diabetes [43, 47, 53]. In addition, it has been previously reported that the oxidation of LDL by glycosylated hemoglobin is not completely blocked by binding to Hp, as occurs in the case of non-glycosylated hemoglobin [43]. Specifically, Hp was shown to inhibit only 10 % of the oxidation induced by glycosylated hemoglobin compared to >90 % of the oxidation of LDL that had not been glycosylated [43]. Thus, as glycosylated hemoglobin levels are elevated in diabetes and the likelihood of LDL oxidation by this pathway is thereby increased, the impaired removal of glycosylated hemoglobin–Hp complexes from the circulation in those with Hp 2-2 may be especially critical [43].

Longitudinal Epidemiologic Studies Demonstrating a Relationship Between the Haptoglobin Genotype and Incident Diabetic Cardiovascular Disease

Several longitudinal studies have provided evidence that the Hp genotype is associated with diabetic cardiovascular complications, although similar associations in persons without diabetes have not been reported (Table 1). Thus, in a matched case–control analysis of the Strong Heart Study, the odds of a cardiovascular event over 6 years of follow-up were almost fivefold (OR=4.96, 95% CI=1.85–3.33) among American Indians with type 2 diabetes and the Hp 2-2 compared to the Hp 1-1 phenotype [55], whereas the intermediate risk associated with the Hp 2-1 phenotype was not significant (OR=1.63, 95% CI=0.74–3.63). In this study, no association between Hp and cardiovascular disease was observed in the non-diabetic population [55]. Furthermore, in the Munich Stent Study, the Hp 2-2 compared to the Hp 1-1 genotype conferred an almost twofold risk (adjusted RR=1.73, 95% CI=1.13–2.65) of the incidence of a major adverse cardiac event within 1 year following percutaneous transluminal coronary angioplasty among 935 consecutive persons with type 2 diabetes treated with oral agents and/or insulin [56]. The risk of a major event increased with the number of Hp 2 alleles, being 21 % in Hp 1-1, 26 % in Hp 2-2, and 31 % in Hp 2-2 individuals (p trend=0.015). The Hp phenotype was also shown to predict 30-day mortality and heart failure among individuals with diabetes and acute myocardial infarction in the Rambam Myocardioal Infarction Outcomes in Diabetes Study, with the Hp 2-2 independently predicting adverse cardiac events compared to the Hp 1-1 phenotype

(adjusted OR=4.9, 95% CI=1.5–15.7) in individuals with diabetes [57]. In this report, the Hp 1-1 phenotype was further associated with a smaller infarct size. Once again, these relationships were only observed in the diabetic population. Finally, in the large (n=3,054) ICARE Trial conducted among individuals with type 2 diabetes [58], cardiovascular disease (cardiovascular death, myocardial infarction, or stroke) incidence did not differ between the Hp 2-1 (2.0 %) and Hp 1-1 (2.1 %) genotypes, although it was increased in those carrying the Hp 2-2 genotype (4.7 %). In this trial, Hp 2-2 was associated with a twofold (adjusted HR=2.3, 95% CI=1.4–3.9) increased risk of cardiovascular disease compared to the Hp 2-1 genotype over an 18-month follow-up [58].

Similar to reports from persons with type 2 diabetes, investigators of the Pittsburgh Epidemiology of Diabetes Complications (EDC) Study of childhood-onset type 1 diabetes demonstrated that the incidence of coronary artery disease increased linearly with the number of Hp 2 alleles, from 15.4 % in Hp 1-1 to 28.3 % in Hp 2-1 and 34.6% in Hp 2-2 (p trend=0.007) [59]. In multivariable Cox models, the Hp 2-2 genotype conferred over a twofold increased risk (HR=2.21, 95% CI=1.05–4.65), although the intermediate risk associated with the Hp 2-1 genotype did not reach statistical significance (HR=1.78, 95% CI=0.84–3.79, p=0.13) [59]. More recently, using data from the Coronary Artery Calcification in Type 1 Diabetes (CACTI) Study, Simpson et al. [60] reported that the Hp 2-2 genotype predicted the development of significant new coronary artery calcification over a 6-year follow-up period among individuals with type 1 diabetes free of coronary artery calcification at study entry. This association was not extended to non-diabetic study participants. As in the EDC Study for coronary events in type 1 diabetes, Hp 2 also appeared to have an allele dose–effect for the development of coronary calcification in the CACTI Study (Hp 2-1: OR=1.72, 95% CI=1.09–2.71; Hp 2-2: OR=2.94, 95% CI=1.87–4.65).

Biologic Mechanisms for a Relationship Between the Hp Genotype and Diabetic Cardiovascular Disease

A major reason the Hp 2-2 genotype appears to be associated with cardiovascular disease in diabetes is thought to relate to the potential for increased oxidative damage caused by the Hp 2-2–hemoglobin complex, which contains redox-active iron and the steady-state plasma concentration of which is elevated compared to the complex formed by Hp 1-1–hemoglobin. A growing body of literature has been accumulating providing supportive data for this hypothesis. Thus, animal experiments have demonstrated an increase in the amount of iron present in the atherosclerotic plaques of transgenic mice with diabetes and the Hp 2-2 genotype, as well as higher levels of oxidative stress and greater macrophage infiltration [61, 62]. Increased iron deposition has also been demonstrated in aortic atherosclerotic plaques of humans with type 2 diabetes and the Hp 2-2 compared to the Hp 1-1 or Hp 2-1 genotype (p=0.02), while no significant increase in iron deposition was observed in the absence of diabetes (p=0.11) [63]. Greater carotid plaque iron deposition was also demonstrated among individuals with diabetes and the Hp 2-2 compared to the Hp 2-1 or Hp 1-1 genotype (p=0.008) in a more recent study of consecutive patients undergoing carotid endarterectomy [64]. Once again, no significant difference was observed in iron deposition by the Hp genotype in non-diabetic plaques (p=0.20). These findings suggest that the impaired clearance of hemoglobin from plaques of individuals with Hp 2-2 may affect the stability of the plaque and lead to atherothrombotic events. These events are exaggerated in the presence of diabetes. Thus, despite an increase in plaque macrophage infiltration, a dramatic reduction has been observed in the proportion of macrophages expressing CD163 in diabetes [46]. Moreover, among individuals with diabetes, the percentage of peripheral blood monocytes expressing CD163 has been reported to be significantly decreased in those carrying the Hp 2-2 compared to the non-Hp 2-2 genotype [46].

Further evidence suggesting increased cardiovascular risk potential with the Hp 2-2 genotype has been provided from in vitro studies demonstrating that the ability of human serum to promote HDL-mediated cholesterol efflux from macrophages decreased linearly with increasing number of Hp 2 alleles of the serum donor [47]. In agreement with epidemiological studies assessing the association between the Hp genotype and cardiovascular outcomes, this effect was restricted to the diabetic state and was not apparent in the absence of diabetes. It was further shown that although glycated Hp 1-1–hemoglobin had no effect on HDL-mediated cholesterol efflux, efflux was markedly inhibited in the case of glycated Hp 2-2–hemoglobin [65]. Significant impairment in HDL-mediated reverse cholesterol transport was also demonstrated in vivo in Hp 2-2 compared to Hp 1-1 diabetic mice [65]. The amount of total lipid peroxides associated with HDL was also found to be increased in individuals with diabetes and the Hp 2-2 compared to the Hp 1-1 genotype [47]. This observation could be due to a decrease in the antioxidant activity of HDL-associated enzymes in Hp 2-2 diabetic individuals and/or an increase in the amount of Hp 2-2–hemoglobin-derived redox-active iron associated with HDL in Hp 2-2 diabetic individuals [44].

Additionally, it has been shown that the observed reduction in the number of macrophages expressing CD163 in the hyperglycemic compared to the normoglycemic state is exacerbated in Hp 2-2 diabetic individuals [61]. Thus, higher plasma concentrations of the Hp–hemoglobin complex in Hp 2-2 diabetic individuals appears to be due to both a less efficient uptake by CD163 as well as a reduced expression of CD163 in the monocytes/macrophages compared to individuals with the Hp 1-1 genotype. As Hp can bind directly to HDL, the elevated plasma concentrations of the Hp–hemoglobin complex in Hp 2-2 diabetic individuals appear to be directly responsible for the increased amount of the complex associated with the HDL of Hp 2-2 diabetic individuals (and mice). Indeed, a greater amount of Hp was associated with HDL in the case of Hp 2 vs. Hp 1 in vitro and in vivo, and the amount of hemoglobin associated with HDL was twofold increased in diabetic individuals with the Hp 2-2 compared to the Hp 1-1 genotype [47]. These mechanistic studies may provide proof of concept for the long proposed hypothesis of dysfunctional HDL in diabetes [66] and may further help explain findings of increased cardiovascular risk among individuals with elevated HDL cholesterol concentrations [67].

An immunomodulatory role for Hp has also been brought to light. As Hp 2-2 is less efficient in the clearance of free hemoglobin following intraplaque hemorrhage, there is greater likelihood for hemoglobin-mediated LDL oxidation [68], which may further lead to B and T cell responses, promoting cellular immunity [69]. In addition, the higher affinity of the Hp 2-2–hemoglobin complex for the CD163 monocyte/macrophage receptor may lead to a greater accumulation of heme iron intracellularly, rendering LDL more susceptible to oxidative stress and promoting an inflammatory response [61]. Moreover, the Hp 1-1–hemoglobin complex, upon binding to CD163, results in a greater production of IL-10 in comparison to the Hp 2-2–hemoglobin complexes [70], which has been suggested to reduce inflammatory cell infiltration and macrophage accumulation in atherosclerotic plaques of mice [61].

Haptoglobin Genotype and Diabetic Kidney Disease

Kidney disease is an established risk factor for the development of cardiovascular complications in both the general population as well as among individuals with diabetes [71]. Indeed, in a meta-analysis of 11 prospective studies conducted in cohorts of type 2 diabetes, microalbuminuria, the earliest indication of kidney disease, was shown to increase the odds of cardiovascular morbidity and mortality twofold [72]. In type 1 diabetes, kidney disease first emerged as a key predictor of cardiovascular events in the mid-1980s, a finding

that has been repeatedly confirmed since [9, 73-76]. In view of the fact that free hemoglobin is taken up by the kidneys in cases where Hp has been depleted, as in hemolysis, a logical question to ask would be whether the Hp genotype is also a determinant of kidney disease risk and whether such a potential relationship is an additional mechanism for the observed association between this polymorphism and an increased risk for cardiovascular events.

The only data to date reporting on the relationship between the Hp genotype and the incidence of diabetic kidney disease have come from the EDC cohort. In this prospective study of type 1 diabetes, investigators demonstrated the presence of an independent association between the Hp 2-2 genotype and early renal function decline and progression to end-stage renal disease [77], although no association was observed with the onset of albuminuria per se. While seemingly conflicting, a pathophysiological mechanism has been proposed, which could explain these findings, and is based on the recognition that renal proximal tubule cells serve as a secondary mechanism for the clearance of the Hp–hemoglobin complex [43]. This mode of clearance is increased for the Hp 2-2–hemoglobin complex since it is not as efficient in promoting uptake by the CD163 receptor. However, increased glomerular filtration of the Hp 2-2–hemoglobin complex leads to a dramatic increase in iron deposition in renal proximal tubule cells, oxidative stress, and hypertrophy [30, 34]. Indeed, significantly increased glomerular and proximal tubular hypertrophy as well as greater deposition of collagen type IV, smooth muscle actin, and increased renal iron were previously shown in Hp 2-2 diabetic mice [34]. Interestingly, supplementation with vitamin E in the latter study appeared to slow renal disease progression in the Hp 2-2 mice, although it did not affect progression in Hp 1-1 mice. These findings suggest that Hp may affect renal function, but not albuminuria per se, and are in agreement with studies showing that reductions in renal function can occur without preceding microalbuminuria in those with diabetes [78-80].

Haptoglobin Genotype and Diabetic Retinopathy

Over 20 years ago, findings from the Framingham Heart and Eye Study suggested that diabetic retinopathy may reflect a generalized microangiopathy that could play an important role in the development of cardiovascular disease [81]. Since, multiple studies have reported an association between the presence of diabetic retinopathy, whether mild or proliferative, and both subclinical as well as clinical cardiovascular events [82-85]. As hyperglycemia-induced oxidative stress in diabetes is central in processes leading to microvascular complications, including retinopathy [86], it would appear possible that risk of retinopathy could differ depending on the Hp genotype. However, results from a recently published study among 98 consecutive adults with type 2 diabetes and either no retinopathy after at least 10 years of diabetes duration or proliferative retinopathy before 10 years following diagnosis showed no increased risk associated with the Hp 2 allele [87]. In this study, an increased risk was shown for neither the development nor the worsening of proliferative retinopathy [87]. To our knowledge, no other studies have to date assessed this association prospectively.

Clinical Trials of Antioxidant Therapy for Cardiovascular Risk Reduction in Diabetes with the Hp 2-2 Genotype

It is evident from findings presented thus far that oxidative stress is at the core of the observed associations between the Hp genotype and the occurrence of cardiovascular events in diabetes. However, despite the wealth and consistency of these findings in different study designs and populations, researchers have been resistant to ask the next logical question, i.e., whether antioxidant supplementation might provide cardiovascular protection specifically to genetically susceptible individuals with diabetes carrying the Hp 2 allele. A considerable

factor in this resistance is likely the numerous failed antioxidant studies over the past decade [88-94]. However, we suggest that the failure to show benefit from antioxidants on the development of cardiovascular disease in these prior studies may have been due to inadequate patient selection. Published reports from three clinical trials thus far have provided exciting results for a protective effect of vitamin E supplementation against the development of cardiovascular disease, specifically in individuals with diabetes and the Hp 2-2 genotype.

The Heart Outcomes Prevention Evaluation (HOPE) Trial of 2,545 women and 6,996 men 55 years of age or older with existing cardiovascular disease or diabetes in addition to one other risk factor assessed the effect of daily supplementation with 400 IU of vitamin E from natural sources vs. placebo on the composite of myocardial infarction, stroke, and death from cardiovascular causes [89]. Overall, during a mean of 4.5 years of follow-up, the trial failed to show any cardiovascular benefit of daily supplementation against the development of either the primary endpoint (RR=1.05, 95% CI=0.95–1.16) or the composite endpoints. However, there was a trend toward a 30 % reduction in the primary endpoint (95% CI=0.45–1.10) as well as a significantly reduced risk of cardiovascular death (RR=0.45, 95% CI=0.23–0.90) and myocardial infarction (RR=0.57, 95% CI=0.33–0.97) with supplement use among those with diabetes and the Hp 2-2 genotype [95].

In the Women's Health Study [90], almost 40,000 healthy women 45 years of age or older were randomly assigned to receive 600 IU natural source vitamin E taken every other day or placebo and aspirin or placebo. Women were followed for a mean of 10.1 years for the composite of the first major cardiovascular event (nonfatal myocardial infarction, nonfatal stroke, or cardiovascular death) and total invasive cancer. A non-significant 7% risk reduction in the primary endpoint was observed with vitamin E supplementation. Supplementation also did not offer protection against incidences of myocardial infarction (p=0.96), stroke (p=0.82), total cancer (p=0.87), breast (p=0.95), lung (p=0.52), or colon (p=0.99) cancer. There was, however, a significant 24 % reduction in cardiovascular death with vitamin E (p=0.03). In this study, analyses among women with diabetes, though limited by the small number of individuals with a diabetes diagnosis and the low event rate stratified by the Hp genotype, showed a non-significant 14 % (95% CI=0.51–1.43) reduction in total cardiovascular disease risk with vitamin E administration among those with the Hp 2-2 genotype and a non-significant increased risk in the non-Hp 2-2 group [96].

In a more recent prospective, double-blinded clinical trial (ICARE), daily supplementation with 400 IU natural D-α-tocopherol acetate in Hp 2-2 diabetic individuals significantly reduced cardiovascular events by about 50 %, bringing the event rate down to a similar rate compared to that observed in the non-Hp 2-2 group [58]. Moreover, although most currently available treatments are now overwhelmed by the effect of statins, it is remarkable that in ICARE, dual therapy with vitamin E and statins provided superior cardiovascular protection compared to statin therapy alone among those with the Hp 2-2 genotype [97], further demonstrating the importance of vitamin E supplementation in this subgroup. The effect of vitamin E in ICARE was also shown to be independent of HbA_{1c} levels as the event rate was reduced from 4.8 to 1.0 % with supplementation among participants with HbA_{1c} below 7 % and the concentration of LDL cholesterol below 100 mg/dL [98]. To alleviate concerns that vitamin E administration may potentially decrease the efficacy of prescribed treatment regimens, Engelen et al. [99] also demonstrated that the efficacy of micronized fenofibrate was not affected by the addition of 400 IU vitamin E daily in patients with type 1 diabetes. Rather, this combination tended to prolong the lag time of the copper-mediated oxidation of non-HDL lipoproteins and amplify the vitamin E-mediated reduction of thiobarbituric acid-reactive substances.

Mechanistically, the protective effect of vitamin E on the prevention of cardiovascular disease in Hp 2-2 diabetic individuals appears to be due, at least in part, to its ability to prevent the oxidative modification of HDL and to preserve the HDL function in this subgroup. In two independent, placebo-controlled, crossover studies, individuals with the Hp 2-2 genotype and diabetes were shown to have a significant improvement in HDL function and a decreased HDL oxidation after vitamin E administration [47, 100]. This favorable effect of vitamin E was not seen in non-Hp 2-2 diabetic individuals [100]. Thus, Asleh et al. [47] demonstrated not only that the half-life of the Hp 2-2–hemoglobin complex is markedly increased in the presence compared to the absence of diabetes but also that the amount of hemoglobin associated with HDL is increased, contains redox-active iron, and results in a marked increase in the amount of lipid peroxides associated with the lipoprotein. In this study, the ability of serum to promote HDL-mediated cholesterol efflux from macrophages was reduced by approximately 30% and 35% in individuals with type 1 diabetes or type 2 diabetes, respectively, and the Hp 2-2 compared to the Hp 1-1 genotype. Remarkably, the addition of vitamin E reduced HDL-associated lipid peroxides and improved cholesterol efflux from macrophages in the Hp 2-2, but not Hp 1-1, individuals with diabetes. Importantly, the withdrawal of vitamin E following the restoration of HDL function resulted in the deterioration of function within 2 months after cessation of supplementation. Similar findings were reported by Farbstein et al. [100] in 59 individuals with type 2 diabetes and either the Hp 2-1 or Hp 2-2 genotype. Thus, supplementation with vitamin E significantly increased HDL function in the Hp 2-2 group, although an unfavorable effect of supplementation was seen in the Hp 2-1 group. Currently, the reason behind the observed detrimental effect of vitamin E in HDL function among those with the Hp 2-1 genotype is not clear.

A potential inconsistency in the hypothesis that antioxidant supplementation provides protection against HDL modification, and thus the development of vascular diabetes complications, was the failure to demonstrate a protective effect of the combination of vitamins C and E against atherosclerosis development in the Women's Antioxidant Vitamin Estrogen (WAVE) Study [101]. WAVE was a prospective, randomized, double-blind trial of 423 postmenopausal women with at least one 15–75 % coronary stenosis [102]. Women were randomly assigned to receive either 0.625 mg/day of conjugated equine estrogen, matching placebo and 400 IU of vitamin E twice daily plus 500 mg of vitamin C twice daily, or placebo. Annualized mean change in the minimum luminal diameter (MLD) from baseline to the concluding angiogram of all qualifying coronary lesions averaged for each patient was the main outcome over a mean of 2.8 years. Contrary to results from all other trials mentioned thus far, a significant benefit in MLD change was seen in those with the Hp 1-1 genotype, which was especially pronounced in diabetes [101]. Conversely, a trend toward a more rapid decrease in MLD with vitamin therapy was observed in Hp 2-2, which was, again, more marked in those with diabetes. It was, however, hypothesized that as glucose-enhanced LDL oxidation is strictly metal iron-dependent [103], it would be possible that supplementation with vitamin C, which increases iron regeneration, may promote LDL oxidation, especially in the presence of the Hp 2-2–hemoglobin complex which contains redox-active iron. Indeed, divergent effects of α-tocopherol and vitamin C on HDL function by Hp genotype in diabetes were demonstrated using in vitro and in vivo studies in transgenic mice [104]. Study investigators showed that vitamin C can neither block HDL oxidation by glycosylated Hp 2-2–hemoglobin complexes nor restore HDL-mediated cholesterol efflux from macrophages, whereas α-tocopherol can favorably affect both. Importantly, the addition of vitamin C to α-tocopherol in this study was shown to diminish the protective effect of vitamin E on HDL function. These results, in addition to the discussion of the different forms of vitamin E below, emphasize that in assessing the effect of antioxidant supplementation on the risk of vascular diabetes complications, selecting a suitable therapeutic agent for the appropriate target population is essential.

Clinical Trials of Vitamin E Supplementation in Relation to Prostate Cancer

Some concerns were recently raised given results of the Selenium and Vitamin E Cancer Prevention Trial (SELECT) in which 400 IU of a synthetic form of vitamin E was associated with an increased risk of prostate cancer [105]. However, these results have to be taken in context with findings from other clinical trials on this topic as SELECT has been the first from a number of studies to show increased risk with vitamin E supplementation. Thus, the Alpha-Tocopherol, Beta-Carotene Cancer Prevention Trial in male smokers reported statistically significant 32 % and 41 % decreases in the incidence of prostate cancer and prostate cancer mortality, respectively, in the α-tocopherol compared to the placebo arm [106]. In the Physician's Health Study II, conducted among US male physicians with a very low proportion of smokers, vitamin E supplementation had no effect on the incidence of prostate cancer or total cancer [107]. Finally, in the Heart Outcomes Prevention and Evaluation—The Ongoing Outcomes (HOPE-TOO) Trial, there was neither significant protection nor harm associated with α-tocopherol supplementation in cancer or cancer deaths [108].

It therefore appears premature to draw conclusions on the effects of vitamin E on prostate cancer, especially since, with the exception of HOPE-TOO, all the aforementioned trials used synthetic forms of the vitamin. Indeed, differences in structure, absorption, and excretion exist between the synthetic and natural forms of α-tocopherol, and natural α-tocopherol comprises only one of the eight different stereoisomers present in the synthetic form [109-111]. Thus, although all forms of vitamin E inhibit in vivo peroxidation, differences among them exist in other biologically relevant roles of the vitamin. In fact, vitamin E is known to modulate signal transduction pathways by binding to tocopherol-associated proteins [112]. However, proteins modulated by the natural vitamin E form may be unresponsive to other stereoisomers and vice versa. Whether such distinctions may carry undesirable consequences is currently unknown. Notably, however, while only the natural form of vitamin E contributes to estimations of the recommended dietary allowance, all forms contribute to toxicity [109, 112].

Concluding Remarks

Cardiovascular disease continues to disproportionately affect individuals with diabetes in the USA and worldwide despite significant advances in prevention efforts and medical care for these patients. It has therefore become apparent that genetic predisposition plays a key role in the development of vascular complications in diabetes, and a functional polymorphism in the Hp gene has been identified as a potential determinant of vascular diabetes complication risk. Indeed, evidence from in vitro animal and human studies relating the Hp 2-2 genotype to cardiovascular disease in diabetes has been robust. Importantly, findings in type 2 diabetes from three clinical trials (only one of which, ICARE, was specifically designed to address this issue) have provided evidence that supplementation with vitamin E has the remarkable potential to reduce cardiovascular events in genetically susceptible individuals with the Hp 2-2 genotype by up to 50 %. Indeed, the relationship of the Hp genotype with the cardiovascular complications of diabetes and the effect modification by vitamin E supplementation exemplifies the idea of pharmacogenomics. Clearly, these findings need to be replicated before this pharmacogenomic paradigm can be proposed as a treatment guideline for individuals with diabetes. It is further imperative that these findings be validated in individuals with type 1 in addition to type 2 diabetes as beyond insulin therapy, limited clinical trial evidence exists for cardiovascular disease prevention in the type 1 population. Undeniably, the ramifications of a replication of the ICARE findings in other type 2 and type 1 diabetes trials are enormous, both because of a significant prevalence of

the Hp 2-2 genotype as well as because of the possibility of a dramatic reduction in risk with a relatively safe and extremely inexpensive regimen.

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Table 1

Studies on the association of the haptoglobin genotype with cardiovascular disease among individuals with diabetes

Reference	Sample size	Follow-up	Outcome	Results	
Nested case–control					
Strong Heart Study [55]	239 individuals with type 2 diabetes and 173 controls, 45–74 years of age	6 years	Incidence of fatal and nonfatal cardiovascular disease	Hp	OR (95% CI)
				1-1	Referent
				2-1	1.63 (0.74–3.63)
				2-2	4.96 (1.85–3.33)
				No differences observed among those without diabetes	
Prospective					
Munich Stent Study [56]	935 consecutive type 2 diabetes patients on oral agents and/or insulin	1 year following percutaneous transluminal coronary angioplasty	Major adverse cardiac events	Hp	Adjusted RR (95% CI)
				1-1	Referent
				2-2	1.73 (1.13–2.65)
Rambam MI Outcomes in Diabetes Study [57]	1,437 patients with acute myocardial infarction (506 with diabetes) and a mean age of 61 years	30 days following admission for an acute myocardial infarction	Composite of mortality and heart failure	Hp	Adjusted OR (95% CI)
				1-1	0.35 (0.15–0.86)
				2-1	1.33 (0.87–2.06)
				2-2	Referent
				No differences observed by Hp in those without diabetes	
Randomized, placebo-controlled clinical trial					
HOPE [95]	3,167 participants (1,078 with diabetes) of the HOPE Trial	4.5 years	Primary composite of cardiovascular death, myocardial infarction, stroke	Outcome	$RR_{Hp\ 2\text{-}2\ vs.\ 1\text{-}1}$ (95% CI)
				Primary composite	0.70 (0.45–1.10)
				CVD death	0.45 (0.23–0.90)
				Nonfatal MI	0.57 (0.33–0.97)
Women's Health Study [96]	721 Caucasian women with diabetes from the Women's Health Study, aged ≥45 years	10 years	Cardiovascular death, nonfatal myocardial infarction, non-fatal	Hp	Vitamin E vs. placebo (% events)

Reference	Sample size	Follow-up	Outcome	Results		
			stroke, or coronary revascularization			
				1-1	12 (21.4%) vs. 10 (17.9%)	
				2-1	37 (23.3%) vs. 30 (17.3%)	
				2-2	31 (21.2%) vs. 31 (23.7%)	
ICARE [58]	2,967 individuals with type 2 diabetes ≥55 years	18 months	Composite outcome of myocardial infarction, stroke, and cardiovascular death	Hp	N	Adjusted HR (95% CI)
				2-1	1,248	1.0
				1-1	285	1.0 (0.4–2.5)
				2-2 Vitamin E	726	1.1 (0.6–2.0)
				2-2 Placebo	708	2.3 (1.4–3.9)