Correction of HDL dysfunction in individuals with Diabetes and the Haptoglobin 2-2 genotype

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Objective. Pharmacogenomics is a key component of personalized medicine. ICARE, a prospective placebo controlled study, recently demonstrated vitamin E could dramatically reduce CVD in individuals with Diabetes Mellitus (DM) and the Haptoglobin (Hp) 2-2 genotype (40% of DM individuals). However, due to the large number of clinical trials which failed to demonstrate benefit from vitamin E coupled with the lack of a mechanistic explanation for why vitamin E should be beneficial only in DM individuals with the Hp 2-2 genotype, enthusiasm for this pharmacogenomic paradigm has been limited. In this study we sought to provide such a mechanistic explanation based on the hypothesis that the Hp 2-2 genotype and DM interact to promote HDL oxidative modification and dysfunction.

Research Design and Methods. Hemoglobin and lipid peroxides were assessed in HDL isolated from DM individuals or mice with the Hp 1-1 or Hp 2-2 genotypes. HDL function was assessed based on its ability to promote cholesterol efflux from macrophages. A crossover placebo controlled study in Hp 2-2 DM humans and in Hp 1-1 and Hp 2-2 DM mice assessed the ability of vitamin E to favorably modify these structural and functional parameters.

Results. Hemoglobin and lipid peroxides associated with HDL were increased and HDL function was impaired in Hp 2-2 DM individuals and mice. Vitamin E decreased oxidative modification of HDL and improved HDL function in Hp 2-2 DM but had no effect in Hp 1-1 DM.

Conclusions. Vitamin E significantly improves the quality of HDL in Hp 2-2 DM individuals.
Pharmacogenomics is a key component of personalized medicine (1). Therapy targeted to a specific patient based on his or her genetically determined pathophysiology responsible for the disease offers the possibility of significantly improving patient care and reducing costs. However, despite the clear public health and economic benefits that would be attained by such an approach this paradigm has not been successfully applied to a common disease.

Cardiovascular disease (CVD) is responsible for 75% of deaths among individuals with Diabetes Mellitus (DM) and yearly expenditures for CVD in DM exceed $200 billion (2). Neither conventional risk factors nor the degree of glycemic control adequately predict which individuals with DM develop CVD, suggesting the existence of genetic susceptibility factors.

A polymorphism in the Haptoglobin (Hp) gene may define which individuals with DM are at greatest risk of CVD. There exist two classes of alleles at the Hp locus denoted 1 and 2 with three possible Hp genotypes 1-1, 2-1, and 2-2 (3). In five independent longitudinal studies performed in ethnically and geographically diverse groups, individuals with the Hp 2-2 genotype and DM were found to have a 2-5 fold increased risk of CVD compared to DM individuals without the Hp 2-2 genotype (4-8). The prevalence of the Hp 2-2 genotype in the DM population in most western countries is approximately 40% making this a common polymorphism.

The Hp polymorphism differs from nearly all polymorphisms being assessed in genome-wide association studies because it is a functional polymorphism (3). Understanding functional differences between the Hp 1 and Hp 2 allelic protein products, particularly in DM, may provide insight into why Hp 2-2 DM individuals have more CVD and how this increased burden of disease might be reduced. The most well understood function of Hp is to bind hemoglobin (Hb) released from erythrocytes (3). Each day over 6 g of Hb is released into the bloodstream due to turnover of erythrocytes and heme iron in this Hb is a powerful oxidizing agent (9,10). Hp which is present in a 400 fold molar excess to free Hb under normal conditions binds Hb, reducing its ability to mediate oxidative modifications and directing its removal from the blood via the monocyte/macrophage CD163 Hp-Hb scavenger receptor (11).

Over five years ago, motivated by in vitro studies demonstrating that the Hp 2-2 protein provides inferior protection against Hb mediated oxidative stress (9,10), coupled with the suggested importance of oxidative stress in DM atherosclerosis (12), we sought to determine if antioxidant therapy might be particularly beneficial to the Hp 2-2 DM cohort. We first tested this hypothesis by examining stored samples from the HOPE study which had failed to demonstrate benefit from vitamin E (13). We found that myocardial infarction and CVD death were reduced by over 40% and 50% respectively in Hp 2-2 DM HOPE participants who received vitamin E (14). In order to prospectively test the hypothesis we initiated a double blind randomized placebo controlled study of vitamin E in 1434 Hp 2-2 DM individuals (ICARE). We found that vitamin E supplementation was associated with a greater than 50% reduction in the combined primary outcome of stroke, myocardial infarction and CV death in Hp 2-2 DM (7).

Enthusiasm for these findings, despite the considerable public health and economic benefits which they suggest, has been muted. Our study comes in the wake of numerous large clinical trials which failed to demonstrate that vitamin E provides any protection against CVD and may be harmful (13,15-20). Further hampering acceptance of this paradigm is the lack of a rational pathophysiologic and pharmacogenomic mechanism to explain why Hp 2-2 DM individuals have an increased risk of CVD and how vitamin E mitigates this risk. In this study we sought to provide a rationale for the pharmacogenomic application of the Hp genotype to prevent CVD in DM by elucidating the unique structural modifications and dysfunctional nature of HDL in Hp 2-2 DM individuals and how these structural and functional changes in HDL are rapidly reversed with vitamin E.
HDL dysfunction and Hp genotype

METHODS

Ethical approval. These studies were approved by the institutional review boards of the Rambam Medical Center and the Technion. All participants provided informed consent.

Human subjects. All studies except where indicated were performed with Type II DM individuals recruited from ICARE (7). The Hp type of participants was determined by gel electrophoresis which has a 100% correspondence with the Hp genotype determined by PCR (21).

Animal studies. The Hp 2 allele is present only in man. All other species have only a Hp 1 allele which is highly homologous with the human Hp 1 allele. We have previously described the construction of a murine Hp 2 allele and the targeting of its insertion by homologous recombination to the murine Hp genetic locus (22). Mice were fed normal chow. DM was produced with streptozotocin at 2 months of age and studied after a DM duration of 1 month.

Measurement of the clearance rate of Hp-Hb in vivo. Hp and Hb were labeled with 125I using chloramine T (23). 125I-Hp-Hb was injected in the tail vein of mice (1 million cpm corresponding to 50 ng) and cpm in serum measured at defined intervals.

Purification of HDL. Ultracentrifuge purified HDL was prepared as previously described (24). Immunopurified HDL was prepared from human or murine serum using a rabbit anti-apoA1 antibody and protein A/G sepharose.

HDL associated lipid peroxides and HDL associated redox active iron. Total lipid peroxides (nmol) associated with HDL were assessed in 1 ug of immunopurified HDL (25). For the assessment of redox active iron associated with HDL the time dependent oxidation of dihydrorhodamine by immunopurified HDL was assessed in the presence and absence of desferroxamine (25).

Assessment of the association of native Hp and Hb with HDL. Hp and Hb were assessed in immunopurified HDL by western blot with either rabbit anti-Hp or anti-Hb antiserum and alkaline phosphatase coupled goat anti-rabbit antiserum for detection.

Cholesterol efflux. Serum from mice or humans treated with placebo or vitamin E was assessed for its ability to promote the efflux of 3H-cholesterol from macrophages (26).

Study drugs. For murine studies, vitamin E was administered in the drinking water at 40mg/kg/day for 30 days beginning one month after onset of DM. For human studies, placebo or vitamin E (400 IU/day of natural source d-alpha tocophorol) capsules were provided in a double blinded format.

Human cross over study design. The study (ClinicalTrials.gov # NCT00314379) was performed in 18 Hp 2-2 DM individuals who were not on antioxidant therapy at baseline (baseline characteristics provided in table 1 of online supplement). Blood was taken at baseline (test 1). Participants were randomly allocated to initially receive vitamin E or placebo for two months after which another blood sample was taken (test 2) and this initial treatment stopped. Two weeks later the participants were crossed over to the other treatment and a blood sample taken after two months of treatment (test 3).

Statistical analysis. All results are reported as mean±SME. Comparison between groups was performed using Student’s t test or ANOVA and the Tukey-Kramer Honestly Significant Difference method for comparisons of means test as appropriate, with a p-value of ≤0.05 considered significant.

RESULTS

The half-life of the Hp 2-2-Hb complex is markedly increased in DM. We sought to test the hypothesis that clearance of Hp-Hb from the plasmatic compartment is both Hp genotype and DM dependent. We tested this hypothesis by injecting 125I-Hp-Hb into Hp 1-1 or Hp 2-2 mice with or without DM. The half-life of Hp 1-1-Hb was approximately 20 minutes with or without DM. The half-life of Hp 2-2-Hb was approximately 50 minutes in mice without DM and over 100 minutes in mice with DM (Table 1).
HDL dysfunction and Hp genotype

Hp is a HDL associated protein in man. Hp has been shown by some but not all investigators to be a HDL associated protein (24,27-29). Critical analysis of these prior studies suggested that the key difference in these studies was in the manner in which the HDL was prepared. We assessed the presence of Hp in human HDL prepared from serum by either ultracentrifugation or immunoabsorption (Figure 1A). We found that Hp is present in the HDL when the HDL is prepared by immunoabsorption but not if the HDL is prepared by ultracentrifugation. While we found that Hp is present in the HDL of all individuals, due to the fact that the Hp 2-2 protein is made up of 3-10 disulfide linked Hp monomers as compared to the Hp 1-1 protein which is made up of only 2 disulfide linked Hp monomers (3), significantly more Hp was detected in the HDL of Hp 2-2 individuals (Figure 1B).

The amount of Hb associated with HDL is increased in Hp 2-2 DM individuals. The binding of Hp to HDL and the high affinity of Hp for Hb suggested that Hp may tether Hb to HDL. Furthermore, the impaired clearance of Hp 2-2-Hb in DM would suggest that there might be more of the complex associated with HDL in Hp 2-2 DM mice or humans. We first investigated this possibility by assessing $^{125}$I-Hp-Hb in the HDL immunoprecipitate and found a dramatic increase, representing over 25% of all injected cpm, in the amount of Hp 2-2-Hb associated with HDL in Hp 2-2 DM mice (Figure 2A). However, in mice genetically deficient for Hp (Hp knockout), no $^{125}$I-Hb was found associated with HDL (zero cpm in HDL immunoprecipitate) demonstrating that Hp is critical for binding of Hb to HDL.

Parallel studies were performed in humans. First, we incubated serum from Hp 1-1, Hp 2-2 or Hp 0 (individuals in whom Hp was not detectable by gel electrophoresis) with $^{125}$I-Hb and assessed the amount of radioactive label in the HDL immunoprecipitate. We found significantly greater Hb associated with HDL in Hp 2-2 serum (Figure 2B).

We then assessed the amount of endogenous Hb associated with HDL in Hp 1-1 and Hp 2-2 mice and humans with and without DM by western blot. We detected substantial amounts of Hb associated with HDL in over 90% of Hp 2-2 DM individuals but failed to find Hb associated with HDL in any Hp 1-1 DM individuals or in any individuals (Hp 1-1 or Hp 2-2) without DM (Figure 3A). Similarly, we found a marked increase in the amount of endogenous Hb associated with HDL in Hp 2-2 DM mice (Figure 3B).

The HDL of Hp 2-2 DM humans contains redox active iron and has increased lipid peroxides. The increased association of the pro-oxidant Hb with HDL in Hp 2-2 DM individuals may result in the increased oxidative modification of HDL associated lipid and proteins and may paradoxically make the HDL a pro-oxidant (30). We assessed oxidation of HDL associated lipids in the HDL of Hp 1-1 and Hp 2-2 DM individuals and found a marked increase in the amount of lipid peroxides in the HDL of Hp 2-2 DM (1.8±0.2 nmol/ug HDL vs. 1.2±0.2 nmol/ug HDL, n=20, p=0.04). HDL from Hp 2-2 DM individuals was also associated with an increased amount of iron capable of mediating oxidation (4.4±0.8 pmol redox active iron/ug HDL vs. 1.8±0.5 pmol redox active iron/ug HDL, n=20, p=0.02).

The HDL in Hp 2-2 DM is dysfunctional. We assessed the ability of serum from Hp 1-1 or Hp 2-2 DM mice or humans with Type I or Type II DM to promote cholesterol efflux from macrophages in vitro. We found a significant 30-40% decrease in HDL function in Hp 2-2 DM as compared to Hp 1-1 DM (Figure 4). No differences were found between Hp 1-1 and Hp 2-2 in the absence of DM or between Hp 1-1 with and without DM (data not shown) (26).

HDL oxidative modification and dysfunction can be corrected in Hp 2-2 DM with vitamin E. We assessed the ability of vitamin E to reduce HDL oxidative modification (HDL associated lipid peroxides) and improve HDL function in Hp 1-1 or Hp 2-2 DM mice. We found that vitamin E had no effect on HDL lipid peroxides or function in Hp 1-1 DM mice. However, vitamin E significantly improved HDL function and reduced HDL lipid peroxides in Hp 2-2 DM mice restoring function and reducing lipid peroxides to levels similar to that found in Hp 1-1 DM (Figure 5).
In humans we assessed the ability of vitamin E to improve HDL function and reduce HDL associated lipid peroxides in Hp 2-2 DM in a crossover study. We found that vitamin E significantly improved HDL function by 30-40% and reduced HDL lipid peroxides by 20-30%. Notably, in this crossover design we found that after vitamin E had restored HDL function and reduced lipid peroxides and the vitamin E was then withdrawn, HDL function deteriorated and HDL associated lipid peroxides increased to levels seen at baseline within two months after cessation of vitamin E supplementation (Figure 6).

DISCUSSION

In this translational study we have provided a pathophysiological and pharmacogenomic rationale as to why vitamin E may provide cardiovascular benefit to individuals with the Hp 2-2 genotype and DM (Figure 7). The main reason why Hp 2-2 DM individuals appear to uniquely derive benefit from vitamin E is that there is substantially more Hb associated with the HDL of Hp 2-2 DM individuals. This key structural difference between HDL in Hp 1-1 and Hp 2-2 DM individuals is the result of an impairment in the CD163 mediated clearance of Hp-Hb in Hp 2-2 DM (23,31).

The association of Hb with HDL results in the oxidative modification of HDL associated proteins and lipids. The loss of function of HDL may be the direct result of its oxidative modification. Hb can oxidize ApoA1 (32) and oxidation of ApoA1 interferes with its ability to promote cholesterol efflux from macrophages (33). Oxidative modification of HDL associated lipids can result in the inactivation of HDL associated antioxidant enzymes such as glutathione peroxidase and paraoxonase (30).

A binding site for Hp on ApoA1 (amino acid residues 141-164) has been identified (27). Interestingly, lecithin acyl transferase (LCAT) whose activity is dependent on its binding to ApoA1 binds to ApoA1 residues 159-170 (34). Hazen and colleagues have shown that nitration or oxidation of Tyr166 in ApoA1 results in an inhibition of the binding of LCAT to ApoA1 (34). We have previously demonstrated a marked decrease in LCAT activity in Hp 2-2 DM individuals (26). We propose that binding of Hp-Hb to a site adjacent to the LCAT binding site may result in the nitration or oxidation (35) of Tyr166 resulting in an impairment in LCAT activity. An impairment in LCAT activity would be expected to impair the maturation of HDL and its ability to promote cholesterol efflux (36). We have indeed found a very tight correlation between LCAT activity and cholesterol efflux in DM individuals (r=0.81, p=0.0002) (26).

The ability of Hb associated with HDL in Hp 2-2 DM individuals to sequester nitric oxide (NO) (37) may have a clinical significance that is of greater importance (38) than the effect of Hb on the function of HDL in reverse cholesterol transport. HDL in Hp 2-2 DM may actually be proatherogenic and prothrombotic by limiting NO bioavailability.

These mechanisms are also relevant to the atherosclerotic plaque. Plaque hemorrhage is recognized as an important determinant of plaque stability (39). The Hp genotype may determine the response to plaque hemorrhage (40). Impaired clearance of Hb in Hp 2-2 DM plaques may lead to oxidative modification of HDL within the plaque and an impairment of its ability to promote reverse cholesterol transport.

The current focus of the medical community towards HDL has been to increase its concentration. The hypothesis presented here may help to explain the dramatically increased CVD risk in patients with Type I DM, despite a usually normal HDL and lipoprotein profile. Moreover, increasing the amount of HDL in individuals in whom the HDL is dysfunctional and potentially proatherogenic may actually be harmful (30). We believe that this is the first demonstration in man that HDL function can be improved in a specific population with vitamin E. However, not all HDL dysfunction can be attributed to Hb mediated oxidation and consequently, not all individuals would be expected to improve the quality of their HDL with vitamin E, as we have demonstrated here with Hp 1-1 DM.

In conclusion we believe that we have provided a pathophysiological and pharmacogenomic rationale as to why vitamin E
may provide benefit to the Hp 2-2 DM cohort. The potential public health and economic benefits from application of this paradigm are enormous. We hope that these findings will encourage testing of this hypothesis in a large scale clinical trial that could result in the establishment of treatment guidelines for individuals with DM.

ACKNOWLEDGEMENTS

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REFERENCES


Table 1. Half-life of the Hp 1-1-Hb and Hp 2-2-Hb complex in non-DM and DM mice and rats

<table>
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<tr>
<th>Animal Strain</th>
<th>DM</th>
<th>N</th>
<th>Hp-Hb complex</th>
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<tbody>
<tr>
<td>Hp 1 mice</td>
<td>-</td>
<td>5</td>
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<tr>
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<tr>
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<tr>
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<td>Rat</td>
<td>-</td>
<td>4</td>
<td>Hp 2</td>
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In the absence of DM the half-life of the Hp 2-Hb complex was significantly increased as compared to the Hp 1-Hb complex in all animals and strains studied (p<0.0001). DM had no effect on the half-life of the Hp 1-Hb complex. However, the half-life of the Hp 2-Hb complex was significantly increased in both Hp 1 and Hp 2 DM mice compared to that observed in Hp 1 mice or Hp 2 mice without DM (p<0.015 comparing the Hp 2-Hb complex in Hp 1 non-DM vs. Hp 1 DM and p<0.0001 comparing the Hp 2-Hb complex in Hp 2 non-DM vs. Hp 2 DM). Moreover, the half-life of the Hp 2-Hb complex was increased to a greater degree in Hp 2 DM mice as compared to Hp 1 DM mice (103±3.9 min vs. 78.2±4.1 min, p<0.005).
Figures

Figure 1.  Hp is an HDL associated protein.

A. Hp is an HDL associated protein in man.  1 ug of HDL prepared from three different individuals with DM by ultracentrifugation (UC) or immunoprecipitation (IP) was subjected to western blot analysis for Hp. Purified Hp 2-1 protein was run as a control to indicate the location of the Hp alpha chains.  M, MW marker.  An immunoreactive band for Hp is seen only in HDL prepared by IP. To confirm equal loading of protein in all lanes the same blot was subsequently developed with an anti-ApoA1 antibody.

<table>
<thead>
<tr>
<th>Patient 1</th>
<th>Patient 2</th>
<th>Patient 3</th>
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<tbody>
<tr>
<td>Hp 2-1</td>
<td>UC</td>
<td>IP</td>
</tr>
<tr>
<td>UC</td>
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</tr>
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<td>IP</td>
<td>M</td>
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B. Increased amount of Hp 2-2 protein associated with human HDL.  Hp was assessed in the HDL immunoprecipitate by western blotting.  Hp alpha chains detected by western are shown.  Purified Hp 2-1 protein (2-1) was run as control to indicate the location of Hp 2-alpha (17kd) and Hp 1-alpha chain (11kd).  Samples denoted 2-2 or 1-1 represent the HDL immunoprecipitate from 6 different individuals with either the Hp 2-2 or Hp 1-1 genotype.
Figure 2. The association of $^{125}\text{Hp-Hb}$ and $^{125}\text{Hb}$ with HDL is Hp genotype and DM dependent

A. Increased association of injected Hp-Hb with HDL in Hp 2-2 DM mice. $^{125}\text{I-Hp-Hb}$ complex (1 million cpm) was injected in the tail vein. The percentage of the injected cpm which coimmunoprecipitated with HDL at all time points after the injection (1-180 minutes) is shown (n=5 for Hp 1-1 and Hp 2-2 non-DM and n=6 for Hp 1-1 and Hp 2-2 DM). There was a significant increase in cpm in the HDL immunoprecipitate of Hp 2-2 DM (p<0.0001 compared to Hp 2-2 non DM). There was no significant difference in cpm in the HDL immunoprecipitate of Hp 1-1 DM as compared to Hp 1-1 non-DM (p=0.24).

B. The ability of $^{125}\text{I-Hb}$ to bind to human HDL in vitro is increased in Hp 2-2 and decreased in Hp 0. $^{125}\text{I-Hb}$ was incubated with serum from individuals with Hp 1-1, Hp 2-2 or Hp 0. $^{125}\text{I-Hb}$ associating with HDL was assessed by immunoprecipitation and the mean±SME for 10 individuals from each of the 3 groups is shown. There was significantly more $^{125}\text{I-Hb}$ associated with HDL using serum from Hp 2-2 individuals as compared to Hp 1-1 individuals (p<0.0001). The amount of $^{125}\text{I-Hb}$ associating with HDL using Hp 0 serum was significantly less than that observed in Hp 1-1 serum (p<0.002). Note that Hp 0 does not indicate that these individuals lack Hp, but rather that the level of Hp is below the level of detection by gel electrophoresis.
Figure 3. Hb is an HDL associated protein in Hp 2-2 DM humans and mice

A. The amount of Hb associated with HDL is increased in Hp 2-2 DM individuals. Western blot for Hb of HDL immunoprecipitate of serum of Hp 1-1 or Hp 2-2 DM individuals. Hb was identifiable in 14/15 DM individuals with the Hp 2-2 genotype and in 0/15 of the DM individuals with the Hp 1-1 genotype. Hb was not found associated with HDL from non DM Hp 1-1 or Hp 2-2 individuals (not shown). Hb indicates purified Hb used as positive control.

B. The amount of Hb associated with HDL is increased in Hp 2-2 DM mice. Western blot for Hb of HDL immunoprecipitate of serum of Hp 1-1 or Hp 2-2 mice with or without DM (D). Hb indicates purified Hb used as positive control.

Figure 4. HDL function is impaired in Hp 2-2 DM mice and humans. Cholesterol efflux from macrophages incubated with serum from Hp 2-2 DM mice and Hp 2-2 humans with Type I DM and with Type II DM is significantly decreased as compared to Hp 1-1 DM mice and Hp 1-1 Type I and Type II DM humans (p=0.0001, n=10 comparing Hp 1-1 vs. Hp 2-2 DM mice; p<0.0006, n=15 comparing Hp 1-1 vs. Hp 2-2 Type I DM individuals; p<0.001, n=30 comparing Hp 1-1 vs. Hp 2-2 Type II DM individuals). Efflux is expressed as the percentage of that obtained for Hp 1-1 DM mice, Type I DM and Type II DM individuals, respectively.
Figure 5. Vitamin E improves HDL function and reduces HDL oxidative modification in Hp 2-2 DM mice but not in Hp 1-1 DM mice.

A. Vitamin E improves the ability of serum of Hp 2-2 DM mice, but not Hp 1-1 DM mice, to promote cholesterol efflux from macrophages. There was a significant difference in efflux elicited by serum from Hp 1-1 and Hp 2-2 DM mice (p=0.002 comparing placebo groups). Vitamin E significantly improved cholesterol efflux in Hp 2-2 DM mice (p=0.0006 comparing Hp 2-2 placebo vs. Hp 2-2 vitamin E). Efflux elicited by the serum of Hp 2-2 DM mice treated with vitamin E was not significantly different from that elicited by Hp 1-1 DM mice. Vitamin E had no effect on efflux in Hp 1-1 DM mice (p=0.29).

![Graph showing cholesterol efflux](image1)

B. Vitamin E reduces HDL associated lipid peroxides in Hp 2-2 DM mice but not in Hp 1-1 DM mice. There was a significant difference in HDL associated lipid peroxides between Hp 1-1 and Hp 2-2 DM mice (p=0.0001). Vitamin E significantly reduced lipid peroxides in Hp 2-2 DM mice (p=0.001 comparing Hp 2-2 placebo vs. Hp 2-2 vitamin E) but had no effect on efflux in Hp 1-1 DM mice (p=0.74 comparing Hp 1-1 placebo vs. Hp 1-1 vitamin E).

![Graph showing HDL lipid peroxides](image2)
Figure 6. Vitamin E improves HDL function and reduces HDL oxidative modification in Hp 2-2 DM humans. Crossover design placebo controlled double blind trial. 18 Hp 2-2 DM individuals divided into two cohorts were randomized to either vitamin E or placebo and treated for 2 months. After a 2 week washout patients were crossed over to the other treatment and treated for an additional 2 months. Blood samples were taken at baseline (test 1), after 2 months of the initial treatment (test 2) and after two months with the second treatment (test 3).

A. Improvement in cholesterol efflux stimulated by Hp 2-2 serum with vitamin E in man. There was a significant improvement in efflux with vitamin E treatment (test 1-test 2 in cohort 1 p=0.004; test 2-test 3 in cohort 2 p=0.04) and no change with placebo treatment (test 1-test 2 in cohort 2, p=0.33). Of note in cohort 1, test 3 is not significantly different from the baseline value demonstrating that even though vitamin E improved HDL function (compare test 1-test 2) after a 2 month period without vitamin E HDL function deteriorated to baseline levels (p=0.13 comparing test 1-test 3 in cohort 1).

B. Reduction in HDL associated lipid peroxides with vitamin E. There was a significant reduction in lipid peroxides with vitamin E treatment (test 1-test 2 in cohort 1 p=0.03; test 2-test 3 in cohort 2 p=0.01) and no change with placebo treatment (test 1-test 2 in cohort 2, p=0.35). Of note in cohort 1, test 3 was not significantly different from the baseline value demonstrating that even though vitamin E reduced lipid peroxides (compare test 1-test 2) 2 months after the vitamin E was stopped lipid peroxides returned to baseline levels (p=0.31 comparing test 1-test 3 in cohort 1).
HDL dysfunction and Hp genotype
Hemoglobin (Hb) released intravascularly from red blood cells (RBC) is rapidly bound by Haptoglobin (Hp) protein to form an Hp-Hb complex. In Hp 2-2 DM individuals the complex is cleared more slowly than in Hp 1-1 DM individuals by the scavenger receptor CD163. The Hp-Hb complex can bind to Apo A1 in HDL, with increased binding of Hp 2-2-Hb occurring due its increased avidity for HDL and its increased plasma concentration. The Hp 2-2-Hb, but not the Hp 1-1-Hb complex, when bound to HDL can produce reactive oxygen species which can oxidize protein (i.e., ApoA1; GPx-glutatione peroxidase; LCAT) and lipid components (cholesterol) of HDL and render the HDL dysfunctional (due to decreased reversed cholesterol transport (RCT) and antioxidant activity) proatherogenic and prothrombotic.