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Vitamin E therapy results in a reduction in HDL function in individuals with Diabetes and the Haptoglobin 2-1 genotype

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Abstract

Objective—Vitamin E provides cardiovascular protection to individuals with Diabetes and the haptoglobin 2-2 genotype but appears to increase cardiovascular risk in individuals with Diabetes and the haptoglobin 2-1 genotype. We have previously demonstrated that the haptoglobin protein is associated with HDL and that HDL function and its oxidative modification are haptoglobin genotype dependent. We set out to test the hypothesis that the pharmacogenetic interaction between the haptoglobin genotype on cardiovascular risk might be secondary to a parallel interaction between the haptoglobin genotype and vitamin E on HDL function.

Research design and methods—Fifty-nine individuals with Diabetes and the haptoglobin 2-1 or 2-2 genotypes were studied in a double-blind placebo controlled crossover design. Participants were treated with either vitamin E (400 IU) or placebo for 3 months and crossed over

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Authors' contribution: DF – cholesterol efflux measurements, CD163 expression, FACS analysis, HDL isolation, GPx measurements, C3 measurements, statistical analysis, SB – Patient recruitment, paper reviewing, contribution to discussion MP – HDL isolation, paper reviewing, contribution to discussion, RA – patient recruitment, paper reviewing, contribution to discussion, HLV – Patient recruitment, paper reviewing, contribution to discussion, OL – Adiponectin and CRP measurements, paper reviewing, contribution to discussion, RA – Lipid peroxides and redox active iron measurements, RML – Hp genotyping, Ido Barkay – patient recruitment, MS – Serum separation, paper reviewing, contribution to discussion, AS – Blinding, statistical analysis of baseline characteristics, paper reviewing, contribution to discussion, SKL – LCAT and $ApoA_1$ nitration measurements, paper reviewing, contribution to discussion, DO – patient recruitment paper reviewing, contribution to discussion, JV – PON_1 measurements paper reviewing, contribution to discussion, HT – PON_1 measurements, paper reviewing, contribution to discussion, MV – statistical analysis, paper reviewing, contribution to discussion, AL – statistical analysis, paper reviewing, contribution to discussion, YA – Hp genotyping, paper reviewing, contribution to discussion, DM – LCAT and ApoE measurements, paper reviewing, contribution to discussion, NA – CD163 expression, paper reviewing, contribution to discussion, NP – patient recruitment, paper reviewing, contribution to discussion, APL – corresponding author.

Potential conflict of interest.

Dr Levy has in the past worked as a consultant for Synvista therapeutics which licensed patents which were the property of Dr Levy's institution that claim that haptoglobin genotype is a determinant of CVD risk in Diabetes.

for an equivalent duration. Serum was collected at baseline and after the completion of each treatment. HDL functionality as well as HDL associated markers of oxidation and inflammation were measured after each interval in HDL purified from the cohort.

Results—Compared to placebo, vitamin E significantly increased HDL function in haptoglobin 2-2 but significantly decreased HDL function in haptoglobin 2-1. This pharmacogenetic interaction was paralleled by similar non-significant trends in HDL associated lipid peroxides, glutathione peroxidase, and inflammatory cargo.

Conclusion—There exists a pharmacogenetic interaction between the haptoglobin genotype and vitamin E on HDL function. (clinicaltrials.gov NCT01113671).

Keywords

Diabetes; Haptoglobin; Vitamin E; Tocopherol; Oxidation; HDL; Cholesterol Efflux

The oxidative hypothesis of atherosclerosis suggests that oxidative modification of lipoproteins may be responsible for the development of atherosclerotic cardiovascular disease (CVD)[1]. This hypothesis is supported by data showing that oxidized lipoproteins can promote foam cell formation and macrophage activation[1], that antioxidants can prevent atherosclerosis in animals[1] and that endogenous levels of antioxidant enzymes are lower in individuals with atherosclerosis[2]. However, attempts to test this hypothesis in placebo controlled clinical trials have shown that vitamin E provides no cardiovascular benefit[3, 4]. One reason why these studies may have failed was the lack of patient selection for antioxidant therapy. One population which might benefit from antioxidant therapy is that defined by a polymorphism in an antioxidant gene which confers inferior antioxidant protection.

The haptoglobin (Hp) protein is an antioxidant due to its ability to neutralize the oxidative activity of hemoglobin (Hb)[5]. In man there exists two classes of alleles at the Hp locus denoted 1 and 2. The protein product of the Hp 2 allele is an inferior antioxidant compared to the Hp 1 allele product[6]. The Hp 2-2 genotype has been associated with a 2-5 fold increased risk of incident CVD in individuals with diabetes (DM) in seven independent studies[7-13].

There appears to exist a pharmacogenetic interaction between the Hp genotype and vitamin E on the development of CVD in individuals with DM[13]. Not only does vitamin E appear to provide substantial cardiovascular benefit to Hp 2-2 DM[9, 13] individuals but it also appears to promote CVD in Hp 2-1 DM individuals[12].

We have recently demonstrated that the Hp protein is associated with HDL. Additionally, we have shown that HDL function and the oxidative modification of HDL are Hp genotype dependent[14, 15]. We hypothesized that the pharmacogenetic interaction of the Hp genotype and vitamin E on CVD might be the result of a parallel interaction of the Hp genotype and vitamin E on HDL function and structure. In order to test this hypothesis we characterized the structure and function of HDL from individuals with DM and the Hp 2-1 or Hp 2-2 genotypes in a double-blind randomized placebo controlled cross-over study.

Materials and Methods

Crossover study design

The study was registered as clinical trial NCT01113671 and approved by the institutional ethics committee of the Rambam Medical Center, Haifa, Israel. All participants provided informed consent. The primary aim of the study was to determine if there existed a

pharmacogenetic interaction between the Hp genotype and vitamin E on HDL function. Subjects were recruited from the ICARE cohort. We initially hoped to achieve a study size of 30 subjects of each Hp type (Hp 1-1, 2-1 and 2-2) in order to provide 80% power to observe a statistically significant interaction between the genotypes assuming that there would be a 20% improvement in HDL function in Hp 2-2 and no effect in the other Hp types. Targeted enrollment was achieved only for Hp 2-1 and Hp 2-2 (42% and 49% of population) but not for Hp 1-1 individuals (9% of population) and therefore analyses are reported only for Hp 2-1 and Hp 2-2. 36 Hp 2-1 and 32 Hp 2-2 DM individuals were consented and enrolled in the study. Participants were randomized to receive either placebo or vitamin E (400IU natural source d-alpha-tocopheryl acetate/day) for 3 months followed by crossover. Serum was collected at baseline and after each treatment and stored at −80°C. Analyses are reported on 31 Hp 2-1 and 28 Hp 2-2 participants who completed the protocol.

Isolation of HDL by affinity chromatography

Serum was diluted in an equal volume of 0.5M NaCl in PBS and loaded onto a polyclonal anti-$ApoA_1$ sepharose column. After washing with PBS the HDL was eluted with tris-glycine (0.1 M, pH 2.5) followed immediately by neutralization with 1M tris, pH 9.0.

Cholesterol efflux

Serum was assessed for its ability to promote the efflux of ^{3}H-cholesterol from J774 A.1 macrophages as previously described[14]. Results presented are normalized to HDL levels and are expressed as the percentage of cholesterol efflux.

Structural analysis of HDL

In this study the structural analysis of HDL consisted of the assessment of several components of the HDL particle: ApoA1, lecithin-cholesterol acyltransferase (LCAT), complement component 3 (C3), lipid peroxides, ApoE and glutathione peroxidase (GPx).

Assessment of HDL associated proteins

C3[16], LCAT[16] and ApoE[16] were assessed in affinity-purified HDL by western blot. GPx-3[17] was assessed in affinity-purified HDL by ELISA .

HDL-associated lipid peroxides and redox active iron

Total lipid peroxides associated with HDL and redox active iron were measured as previously described[14].

Serum CRP and adiponectin

Serum high sensitivity C-reactive protein (hs-CRP) and adiponectin levels were assessed by ELISA .

CD163 expression

Expression of CD163 by peripheral blood monocytes (PBMs) was examined by flow cytometry. Cells were treated with APC conjugated α-CD14 and PE conjugated α-CD163 antibodies and analysis was performed on a CyAN ADP analyzer.

Serum Lipid Profile and $ApoA_1$

HDL, total cholesterol and $ApoA_1$ were measured as previously described[18]. Nitration of ApoA1 was determined by western blot with results normalized to ApoA1.

Serum Paraoxonase 1 (PON1) activity

Catalytic activities of PON1 was determined by using 5-(thiobutyl)-butyrolactone as substrate[19].

Statistical analysis

Results are reported as means ±SE. Comparison of parametric values between groups was performed using Student's t-test or paired t-test as appropriate, with a p of ≤0.05 considered significant. Non-parametric values were compared with chi-square test. Interaction testing was performed as described in an online appendix. Briefly, we computed the change in efflux and relative change in efflux (change divided by baseline). Modeling was done with two observations for each patient (placebo and vitamin E) We computed the least square means with 95% confidence intervals with the PROC MIXED procedure in SAS 9.2.

Results

Baseline characteristics of Hp 2-1 and Hp 2-2 participants

Study participants with Hp 2-1 and Hp 2-2 genotypes did not differ in their baseline demographics and in the characteristics or management of their DM (Table 1 online supplement). At baseline, both $ApoA_1$ and serum stimulated cholesterol efflux (RCT) were significantly increased in Hp 2-1 as compared to Hp 2-2 study participants ($ApoA_1$: Hp 2-1, 156.8±3.4 vs. Hp 2-2, 146.7 ±3.7 p=0.05; RCT: Hp 2-1, 15.4±1.1% vs Hp 2-2, 11.8±0.8%, p=0.01) (Table 2 online supplement).

Vitamin E improves RCT in Hp 2-2 but inhibits RCT in Hp 2-1

While in the Hp 2-2 cohort vitamin E was associated with a significantly higher RCT as compared to placebo (12.1±0.81% vitamin E vs. 11.1±0.64% placebo, n=28; P=0.05); in the Hp 2-1 cohort vitamin E was associated with a significantly lower RCT as compared to placebo (13.9±1.1% vitamin E vs. 15.4±1.0% placebo, n=31; P=0.04). There was a statistically significant interaction between the Hp genotype (Hp 2-1 vs Hp 2-2) and vitamin E on RCT (p=0.006). There was no significant effect of vitamin E on LCAT, $ApoA_1$ or HDL in Hp 2-1 or Hp 2-2 participants (Tables 1 and 2).

Vitamin E reduces HDL associated lipid peroxides in Hp 2-2 but not Hp 2-1

Oxidative modification has been proposed to be the mechanism by which HDL is rendered dysfunctional and antioxidant therapy appears to restore HDL functionality[14, 20]. We therefore sought to determine if the interaction between vitamin E and Hp genotype on RCT might be explained by a differential effect of vitamin E on HDL oxidative modification in Hp 2-1 and Hp 2-2. Vitamin E resulted in a 50% reduction in HDL associated lipid peroxides in Hp 2-2 (0.55±0.10 nmol Vitamin E vs. 1.07±0.19 nmol placebo; p=0.003) but had no effect in Hp 2-1. Furthermore, we observed a trend showing a greater than 20% decrease in nitration of $ApoA_1$ in Hp 2-2 with vitamin E, while in Hp 2-1 vitamin E had no effect on this parameter (Tables 1 and 2).

In order to determine why vitamin E reduced HDL lipid peroxidation in Hp 2-2 but not Hp 2-1 we investigated the effect of vitamin E on the mass or activity of the antioxidant proteins GPx-3 and paraoxonase known to be associated with HDL[16, 17], as well as the amount of redox-active nontransferrin-bound iron which has previously been implicated in the oxidation of HDL[14] (Tables 1 and 2). While the effect of vitamin E did not reach statistical significance for any of these measurements we did observe that vitamin E was associated with an approximately 50% increase in HDL associated glutathione peroxidase and a 25%

reduction in redox active iron in Hp 2-2 while there was a 3-4 fold decrease in HDL associated glutathione peroxidase (p=0.06) and no change in redox active iron in Hp 2-1.

Vitamin E and CD163

The increase in redox active iron in Hp 2-2 DM has been attributed to the impaired clearance of Hp 2-2-Hb by the CD163 Hp-Hb receptor[21, 22]. Surface expression of CD163 is regulated by oxidative stress and hyperglycemia[22, 23]. We sought to determine if the decrease in redox-active iron in Hp 2-2 DM individuals who received vitamin E may have been associated with an increase in CD163 expression on PBMs. We observed a trend showing a greater than 50% increase in CD163 expression in PBMs of Hp 2-2 individuals receiving vitamin E. Vitamin E appeared to be associated with a 50% reduction in CD163 expression in Hp 2-1 individuals (Tables 1 and 2).

Vitamin E decreases association of the inflammatory marker C3 with HDL in Hp 2-2 but not in Hp 2-1

In addition to functioning to promote RCT and to prevent the oxidation of LDL, HDL has been described as having an anti-inflammatory function[20]. However, pro-inflammatory biomarkers such as C3 have been associated with dysfunctional HDL in individuals with CVD[16]. We sought to determine whether vitamin E would decrease the association of C3 with HDL in Hp 2-2. We found that vitamin E treatment was associated with a borderline significant decrease in C3 associated HDL in Hp 2-2 individuals (1.07±0.09 vitamin E vs. 1.34±0.20 placebo; n=25; p=0.09) but had no effect on the association of C3 with HDL in Hp 2-1 (1.08±0.07 vs. 1.08±0.07, n=30; p=0.99). This effect of vitamin E on a HDL associated inflammatory marker was not associated with an overall change in serum markers of inflammation such as CRP and adiponectin (Table 1 and 2).

Discussion

We have provided a plausible mechanism for the divergent effects of vitamin E therapy on cardiovascular risk in DM individuals with the Hp 2-1 and Hp 2-2 genotypes. While vitamin E improves HDL function in Hp 2-2 DM individuals it decreases HDL function in Hp 2-1 DM individuals. Structural analysis of HDL in study participants suggested a similar interaction in which vitamin E appeared to result in a favorable although non-statistically significant change in a number of HDL associated oxidative and inflammatory markers in Hp 2-2 individuals (the decrease in HDL associated lipid peroxides was statistically significant) while producing no beneficial effect on these markers in Hp 2-1 individuals. This study therefore supports the concept that there exists a pharmacogenetic interaction between the Hp genotype and vitamin E in individuals with DM[12, 13].

The cholesterol efflux assay used in this study utilizing whole serum from patients is virtually identical to that used recently by Khera and colleagues[24] assessing the direct relationship between cholesterol efflux capacity and atherosclerotic burden. This assay integrates total efflux mediated by several pathways which have been shown to be important for the efflux of cholesterol from macrophages (i.e., ATP-binding cassette transporter A1 [ABCA1] and G1 [ABCG1], scavenger receptor B1, and aqueous diffusion)[25]. The effect of Hp genotype on these mediators of cholesterol efflux is unknown although we have previously reported a relationship between LCAT activity and Hp genotype[15].

The favorable effects of vitamin E on HDL structure and lipid peroxidation described here are most likely due to the inhibition of oxidative modifications mediated by Hp 2-2-Hb associated with $ApoA_1$[14]. Hp has been demonstrated to bind to $ApoA_1$ and this Hp can tether Hb to HDL[14, 26]. Hp 2-2 is inefficient in blocking the redox activity of Hb derived

iron and therefore in Hp 2-2 individuals HDL becomes the carrier of a cargo which is pro-oxidative[14, 27].

The detrimental effects of vitamin E on HDL structure in Hp 2-1 individuals may be due to an overshoot in the suppression of oxidative stress by vitamin E. Suppression of oxidative stress to too great a degree may be deleterious. Several groups have demonstrated both in animals and in man a down-regulation of protective antioxidant enzymes in response to high dose antioxidant supplementation[28, 29]. This down-regulation may paradoxically increase the susceptibility of these individuals to acute increases in oxidative stress (as with wide swings in hyperglycemia). The implications of this hypothesis are that it may be possible to demonstrate that in Hp 2-1 individuals a lower dose of vitamin E may have beneficial effects on CVD.

We believe that the changes we report here on HDL function are biologically significant relevant with regards to vitamin E effects on CVD risk. Modest changes in cholesterol efflux identical to what we report here were shown to be directly associated with atherosclerotic burden and CVD[24]. Khera and colleagues demonstrated that cholesterol efflux is related to intima media thickness and the likelihood of angiographic coronary artery disease independent of HDL cholesterol[24]. The difference in efflux between the groups with and without CVD in that study was less than 10%[24]. The changes in efflux with vitamin E seen in our study were 10% (11% reduction in Hp 2-1 and 9% increase in Hp 2-2).

The public health and economic implications of the pharmacogenetic interaction between the Hp type and vitamin E on CVD are profound[13]. Implementation of a pharmacogenetic algorithm for DM patients in which all individuals with DM and the Hp 2-2 genotype would receive vitamin E cannot be achieved without an additional clinical trial testing this hypothesis. We hope the mechanistic data presented here will help to increase interest for such a trial.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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References

1. Witztum JL. The oxidation hypothesis of atherosclerosis. Lancet. 1994; 344:793–795. [PubMed: 7916078]
2. Flores-Mateo G, Carrillo-Santisteve P, Elosua R, Guallar E, Marrugat J, Bleys J, Covas MI. Antioxidant enzyme activity and coronary heart disease: meta-analyses of observational studies. Am J Epidemiol. 2009; 170:135–147. [PubMed: 19465742]
3. Vivekananthan DP, Penn MS, Sapp SK, Hsu A, Topol EJ. Use of antioxidant vitamins for the prevention of cardiovascular disease: meta-analysis of randomised trials. Lancet. 2003; 361:2017–2023. [PubMed: 12814711]
4. Yusuf S, Dagenais G, Pogue J, Bosch J, Sleight P, The Heart Outcomes Prevention Evaluation Study Investigators. Vitamin E supplementation and cardiovascular events in high-risk patients. N Engl J Med. 2000; 342:154–160. [PubMed: 10639540]
5. Bowman BH, Kurosky A. Haptoglobin: the evolutionary product of duplication, unequal crossing over, and point mutation. Adv Hum Genet. 1982; 12:189–261. 453–184. [PubMed: 6751044]

6. Melamed-Frank M, Lache O, Enav BI, Szafranek T, Levy NS, Ricklis RM, Levy AP. Structure-function analysis of the antioxidant properties of haptoglobin. Blood. 2001; 98:3693–3698. [PubMed: 11739174]
7. Costacou T, Ferrell RE, Orchard TJ. Haptoglobin genotype: a determinant of cardiovascular complication risk in type 1 diabetes. Diabetes. 2008; 57:1702–1706. [PubMed: 18332093]
8. Levy AP, Hochberg I, Jablonski K, Resnick HE, Lee ET, Best L, Howard BV. Haptoglobin phenotype is an independent risk factor for cardiovascular disease in individuals with diabetes: The Strong Heart Study. J Am Coll Cardiol. 2002; 40:1984–1990. [PubMed: 12475459]
9. Milman U, Blum S, Shapira C, Aronson D, Miller-Lotan R, Anbinder Y, Alshiek J, Bennett L, Kostenko M, Landau M, Keidar S, Levy Y, Khemlin A, Radan A, Levy AP. Vitamin E supplementation reduces cardiovascular events in a subgroup of middle-aged individuals with both type 2 diabetes mellitus and the haptoglobin 2-2 genotype: a prospective double-blinded clinical trial. Arterioscler Thromb Vasc Biol. 2008; 28:341–347. [PubMed: 18032779]
10. Roguin A, Koch W, Kastrati A, Aronson D, Schomig A, Levy AP. Haptoglobin genotype is predictive of major adverse cardiac events in the 1-year period after percutaneous transluminal coronary angioplasty in individuals with diabetes. Diabetes Care. 2003; 26:2628–2631. [PubMed: 12941730]
11. Suleiman M, Aronson D, Asleh R, Kapeliovich MR, Roguin A, Meisel SR, Shochat M, Sulieman A, Reisner SA, Markiewicz W, Hammerman H, Lotan R, Levy NS, Levy AP. Haptoglobin polymorphism predicts 30-day mortality and heart failure in patients with diabetes and acute myocardial infarction. Diabetes. 2005; 54:2802–2806. [PubMed: 16123372]
12. Blum S, Vardi M, Levy NS, Miller-Lotan R, Levy AP. The effect of vitamin E supplementation on cardiovascular risk in diabetic individuals with different haptoglobin phenotypes. Atherosclerosis. 2010; 211:25–27. [PubMed: 20223458]
13. Blum S, Vardi M, Brown JB, Russell A, Milman U, Shapira C, Levy NS, Miller-Lotan R, Asleh R, Levy AP. Vitamin E reduces cardiovascular disease in individuals with diabetes mellitus and the haptoglobin 2-2 genotype. Pharmacogenomics. 2010; 11:675–684. [PubMed: 20415560]
14. Asleh R, Blum S, Kalet-Litman S, Alshiek J, Miller-Lotan R, Asaf R, Rock W, Aviram M, Milman U, Shapira C, Abassi Z, Levy AP. Correction of HDL dysfunction in individuals with diabetes and the haptoglobin 2-2 genotype. Diabetes. 2008; 57:2794–2800. [PubMed: 18599520]
15. Asleh R, Miller-Lotan R, Aviram M, Hayek T, Yulish M, Levy JE, Miller B, Blum S, Milman U, Shapira C, Levy AP. Haptoglobin genotype is a regulator of reverse cholesterol transport in diabetes in vitro and in vivo. Circ Res. 2006; 99:1419–1425. [PubMed: 17082477]
16. Vaisar T, Pennathur S, Green PS, Gharib SA, Hoofnagle AN, Cheung MC, Byun J, Vuletic S, Kassim S, Singh P, Chea H, Knopp RH, Brunzell J, Geary R, Chait A, Zhao XQ, Elkon K, Marcovina S, Ridker P, Oram JF, Heinecke JW. Shotgun proteomics implicates protease inhibition and complement activation in the antiinflammatory properties of HDL. J Clin Invest. 2007; 117:746–756. [PubMed: 17332893]
17. Chen N, Liu Y, Greiner CD, Holtzman JL. Physiologic concentrations of homocysteine inhibit the human plasma GSH peroxidase that reduces organic hydroperoxides. J Lab Clin Med. 2000; 136:58–65. [PubMed: 10882228]
18. Nasser NJ, Kaplan M, Nevo E, Aviram M. Lipid profile and serum characteristics of the blind subterranean mole rat, Spalax. PLoS One. 2009; 4:e4528. [PubMed: 19229331]
19. Khersonsky O, Tawfik DS. Chromogenic and fluorogenic assays for the lactonase activity of serum paraoxonases. Chembiochem. 2006; 7:49–53. [PubMed: 16329153]
20. Van Lenten BJ, Hama SY, de Beer FC, Stafforini DM, McIntyre TM, Prescott SM, La Du BN, Fogelman AM, Navab M. Anti-inflammatory HDL becomes pro-inflammatory during the acute phase response. Loss of protective effect of HDL against LDL oxidation in aortic wall cell cocultures. J Clin Invest. 1995; 96:2758–2767. [PubMed: 8675645]
21. Asleh R, Marsh S, Shilkrut M, Binah O, Guetta J, Lejbkowicz F, Enav B, Shehadeh N, Kanter Y, Lache O, Cohen O, Levy NS, Levy AP. Genetically determined heterogeneity in hemoglobin scavenging and susceptibility to diabetic cardiovascular disease. Circ Res. 2003; 92:1193–1200. [PubMed: 12750308]

22. Levy AP, Purushothaman KR, Levy NS, Purushothaman M, Strauss M, Asleh R, Marsh S, Cohen O, Moestrup SK, Moller HJ, Zias EA, Benhayon D, Fuster V, Moreno PR. Downregulation of the hemoglobin scavenger receptor in individuals with diabetes and the Hp 2-2 genotype: implications for the response to intraplaque hemorrhage and plaque vulnerability. Circ Res. 2007; 101:106–110. [PubMed: 17525367]

23. Timmermann M, Hogger P. Oxidative stress and 8-iso-prostaglandin F(2alpha) induce ectodomain shedding of CD163 and release of tumor necrosis factor-alpha from human monocytes. Free Radic Biol Med. 2005; 39:98–107. [PubMed: 15925282]

24. Khera AV, Cuchel M, de la Llera-Moya M, Rodrigues A, Burke MF, Jafri K, French BC, Phillips JA, Mucksavage ML, Wilensky RL, Mohler ER, Rothblat GH, Rader DJ. Cholesterol efflux capacity, high-density lipoprotein function, and atherosclerosis. N Engl J Med. 2011; 364:127–135. [PubMed: 21226578]

25. de la Llera-Moya M, Drazul-Schrader D, Asztalos BF, Cuchel M, Rader DJ, Rothblat GH. The ability to promote efflux via ABCA1 determines the capacity of serum specimens with similar high-density lipoprotein cholesterol to remove cholesterol from macrophages. Arterioscler Thromb Vasc Biol. 2010; 30:796–801. [PubMed: 20075420]

26. Spagnuolo MS, Cigliano L, D'Andrea LD, Pedone C, Abrescia P. Assignment of the binding site for haptoglobin on apolipoprotein A-I. J Biol Chem. 2005; 280:1193–1198. [PubMed: 15533931]

27. Asleh R, Guetta J, Kalet-Litman S, Miller-Lotan R, Levy AP. Haptoglobin genotype- and diabetes-dependent differences in iron-mediated oxidative stress in vitro and in vivo. Circ Res. 2005; 96:435–441. [PubMed: 15662028]

28. Ristow M, Zarse K, Oberbach A, Kloting N, Birringer M, Kiehntopf M, Stumvoll M, Kahn CR, Bluher M. Antioxidants prevent health-promoting effects of physical exercise in humans. Proc Natl Acad Sci U S A. 2009; 106:8665–8670. [PubMed: 19433800]

29. Rainwater DL, Mahaney MC, VandeBerg JL, Wang XL. Vitamin E dietary supplementation significantly affects multiple risk factors for cardiovascular disease in baboons. Am J Clin Nutr. 2007; 86:597–603. [PubMed: 17823422]

Table 1

Effect of vitamin E on lipid profile and various markers of oxidation, inflammation and HDL structure and function, in Hp 2-2 diabetic humans

Marker	Vitamin E		Placebo		N	P_{value}
	Average	SEM	Average	SEM		
Cholesterol Efflux (%)	12.1	0.81	11.1	0.64	28	P=0.05
Lipid Peroxides (nmol/μg HDL)	0.549	0.104	1.070	0.190	28	P=0.003
Complement C3 Deposition (ratio to baseline)	1.070	0.0929	1.344	0.197	25	P=0.09
Labile Plasma Iron (μM)	0.152	0.0331	0.211	0.0407	28	P = 0.12
$ApoA_1$ nitration (ratio to HDL-$ApoA_1$)	0.416	0.0586	0.527	0.129	29	P = 0.18
HDL associated LCAT (ratio to baseline)	1.082	0.0789	1.072	0.0854	27	P=0.88
HDL associated GPx-3 (ng/μg HDL)	5.25×10^{-3}	0.975×10^{-3}	3.57×10^{-3}	1.44×10^{-3}	10	P=0.24
HDL associated ApoE (ratio to baseline)	2.102	0.290	2.242	0.378	25	P = 0.70
CD163 Expression (%)	16.636	4.097	11.688	3.490	18	P = 0.29
Serum PON_1 Activity	18.149	2.285	17.606	1.687	15	P=0.55
Serum CRP (ng/ml)	1053.495	250.663	1090.625	215.991	28	P = 0.87
Serum adiponectin (ng/ml)	5.765	0.901	5.429	0.829	20	P = 0.28
$ApoA_1$ (blood) (mg/dL)	147.286	3.850	144.393	3.273	28	P = 0.13
HDL (mg/dL)	43.750	1.849	42.857	1.592	28	P = 0.30
Total Cholesterol (mg/dL)	157.643	5.824	150.143	4.759	28	P = 0.19
Non HDL Cholesterol (mg/dL)	114.250	6.001	107.286	5.118	28	P = 0.20

Complement C3 – Complement component 3, LCAT – lecithin cholesterol acyltransferase, GPx-3 – Glutathione Peroxidase-3, PON_1 – Paraoxonase-1, CRP – C-Reactive Protein.

Table 2

Effect of vitamin E on lipid profile and various markers of oxidation, inflammation and HDL structure and function, in Hp 2-1 diabetic humans

Marker	Vitamin E		Placebo		N	P_{value}
	Average	SEM	Average	SEM		
Cholesterol Efflux (%)	13.9	1.09	15.4	1.00	31	P=0.04
Lipid Peroxides (nmol/μg HDL)	1.065	0.156	1.071	0.174	31	P=0.95
Complement C3 deposition (ratio to baseline)	1.083	0.0816	1.084	0.0723	30	P = 0.99
Labile Plasma Iron (μM)	0.250	0.0486	0.241	0.0464	31	P = 0.75
$ApoA_1$ nitration (ratio to HDL-$ApoA_1$)	0.317	0.0405	0.312	0.0349	31	P = 0.84
HDL associated LCAT (ratio to baseline)	1.351	0.173	1.338	0.168	25	P=0.93
HDL associated GPx-3 (ng/μg HDL)	2.09×10^{-3}	0.79×10^{-3}	7.33×10^{-3}	2.05×10^{-3}	10	P=0.07
HDL associated ApoE (ratio to baseline)	2.255	0.444	2.824	0.770	23	P=0.21
CD163 expression (%)	14.672	4.285	22.063	6.410	13	P = 0.33
Serum PON_1 Activity	20.019	2.542	19.955	2.442	15	P = 0.96
Serum CRP (ng/ml)	1207.948	264.883	1025.097	163.545	27	P=0.31
Serum Adiponectin (ng/ml)	5.081	0.721	5.393	0.916	23	P = 0.45
$ApoA_1$ (blood) (mg/dL)	153.839	3.382	151.497	4.559	31	P = 0.57
HDL (mg/dL)	47.613	1.916	46.903	2.229	31	P = 0.81
Total Cholesterol (mg/dL)	165.387	5.879	160.323	6.186	31	P = 0.23
Non HDL Cholesterol (mg/dL)	117.774	6.332	113.419	6.135	31	P = 0.24

Complement C3 – Complement component 3, LCAT – lecithin cholesterol acyltransferase, GPx-3 – Glutathione Peroxidase-3, PON_1 – Paraoxonase-1, CRP – C-Reactive Protein.