Clearance of the Haptoglobin 2-2-Hemoglobin Complex is Impaired in Diabetes Mellitus Resulting in a Modification of HDL Structure and Defective Reverse Cholesterol Transport

Rabea Asleh, Rachel Miller-Lotan, <u>Andrew P. Levy</u>

Diabetic Cardiovascular Research, Technion Faculty of Medicine, Technion Israel Institute of Technology, Haifa, Israel

Background. Haptoglobin (Hp) plays a key role in clearing extracorpuscular hemoglobin (Hb). Two common alleles exist at the Hp locus (1 and 2). We recently demonstrated that reverse cholesterol transport is impaired in individuals with Diabetes Mellitus (DM) and the Hp 2-2 genotype which may explain the increased incidence of CVD in this population. We sought to test the hypothesis that clearance of the Hp 2-Hb complex is slower in DM allowing more complex to bind to HDL thereby resulting in increased oxidative modification of HDL and inhibition of reverse cholesterol transport.

Methods and Results. Injection of I^{125}- Hp 1 or Hp 2-Hb complexes into non-DM mice demonstrated that the half-life of the Hp 2-Hb complex was 2-3 fold longer than the Hp 1-Hb complex (57.8±2.8 vs. 20.4±1.7 min). Moreover, in DM the half-life of the Hp 2-Hb complex was doubled while the half-life of the Hp 1-Hb complex was unchanged (103±3.9 vs. 18.6±1.8 min). Coimmunoprecipitation studies demonstrated that over 25% of the injected Hp 2-Hb complex was associated with HDL in DM mice representing a greater than 10 fold increase compared to Hp 1-Hb complex in non-DM mice. Reverse cholesterol transport was impaired by DM in Hp 2 mice but this impairment was prevented by vitamin E supplementation to these mice.

Conclusions. These data may explain why the Hp 2 genotype promotes less efficient reverse cholesterol transport in DM and suggests that strategies targeted to decrease oxidation of HDL by the Hp 2-Hb complex may improve HDL function.

Key Words: Haptoglobin, Hemoglobin, Diabetes Mellitus, Atherosclerosis, Cardiovascular Disease, HDL cholesterol, Oxidant stress.

Lifestyle Intervention in Obese Arab Women at High Risk for Diabetes and Cardiovascular Disease: Preliminary Results

Nuha Zaidan-Youniss [2,4], Flora Lubin [1], Ahmed Atamna [2], Daphna Gofer [3], Gershon Alpert [2], Ofra Kalter-Leibovici [1,4]

[1] *Unit of Cardiovascular Epidemiology, Gertner Institute for Epidemiology and Health Policy Research, Sheba Medical Center, Ramat Gan,* [2] *Clalit Health Services, Hadera,* [3] *Information and Computer Unit, Gertner Institute for Epidemiology and Health Policy Research, Sheba Medical Center, Ramat Gan,* [4] *Epidemiology & Preventive Medicine, Faculty of Medicine, Tel-Aviv University, Tel Aviv, Israel*

Background: Arab women in Israel are at high risk for obesity, diabetes and cardiovascular disease (CVD). Lifestyle intervention proved successful in preventing diabetes and modifying cardiovascular risk in Western populations might fail in conservative societies.

Aims: To design, implement and evaluate a culture-sensitive lifestyle intervention in obese Arab women at high risk for diabetes and CVD.

Study Design: Randomized clinical trial.

Intervention: A total of 204 women, 35-54 years old, were allocated to either the intensive or to the moderate (control) lifestyle intervention arm.

❋ <u>Intensive lifestyle intervention</u>: included dietary and physical education counseling in weekly sessions.

❋ <u>Moderate lifestyle intervention</u>: included two educational sessions at baseline, individual dietary counseling every 6 months, and provision of written educational material on lifestyle modification.

Endpoints: Weight reduction and change in risk factors associated with diabetes and CVD.

Results: Preliminary results in 178 women who completed 6 months' follow-up showed greater reduction in body weight and HOMA-IR in women allocated to the intensive lifestyle intervention arm compared to women in the moderate intervention arm (see Table). Drop-out rate at 6 months was 21%.

	Type of Lifestyle Intervention (Study Arm)		P
	Intensive *N=93*	Moderate *N=85*	
Baseline weight (kg)*	87.7 ± 9.6	86.9 ± 8.0	0.56
% weight change at 6 months*	-4.0 ± 5.5	-0.4 ± 4.8	<0.001
Change in HOMA-IR at 6 months**	-0.65 (-5.30-3.04)	-0.15 (-2.11-1.91)	<0.001

*-Mean ± SD
**-Median (range)

Summary and Conclusion: A culture-sensitive lifestyle intervention can lead to significant moderate weight reduction and improve insulin sensitivity in obese Arab women.

Tight Diabetic Glycemic Control Reduces the Risk of Cardiovascular Disease Only in Individuals with the Haptoglobin 2-2 Genotype

Shany Blum[1], Uzi Milman[2], Chen Shapira[2], Rachel Miller-Lotan[1], Yefim Anbinder[1], Junia Alshiek[1], Lawrence Bennett[2], Maria Kostenko[2], Michele Landau[5], Shlomo Keidar[3], Yishai Levy[3], Alexander Khemlin[2], Arman Radan[2], Andrew Levy[1]

[1] Laboratory of Vascular Medicine, Faculty of Medicine, Technion-Israel Institute of Technology, [2] Haifa and Western Galilee District, Clalit Health Services, [3] Internal Medicine, [4] Cardiology Department, Rambam Medical Center, Haifa, [5] PharmaBrains Israel, Tel Aviv, Israel

Background. The Haptoglobin gene is polymorphic in man with two classes of alleles denoted 1 and 2. Several cross sectional and retrospective analysis have suggested that the Haptoglobin genotype may be a major determinant of susceptibility to diabetic CVD. We sought to examine this relationship in a prospective population based study.

Methods. We recruited 3000 individuals, age ≥55 years with DM from primary health care clinics of the Clalit Health Services and obtained Haptoglobin genotype on all individuals. The prevalence of CVD at baseline was 25%. Patients were followed for 18 months, for the primary composite outcome of the study which was incident non-fatal myocardial infarction, stroke and CV death.

Results. We found that the Haptoglobin 2-2 genotype was associated with a highly significant increase in the incidence of myocardial infarction, stroke and CV death. Moreover, after stratification of patients by baseline HbA1c to those above and below 7.0, as currently recommended by the AHA/ADA, only in Haptoglobin 2-2 individuals was poor glycemic control found to be associated with an increased risk of major cardiovascular events (2.2% vs. 4.7% respectively, p=0.027 by log-rank).

Conclusions. Optimal utilization of health care resources for risk factor modification should be focused on DM individuals with the Haptoglobin 2-2 genotype. Benefit from tight glycemic control only in a subset of the DM cohort defined by the Haptoglobin 2-2 genotype may explain the inability to show a benefit from tight glycemic control on reducing cardiovascular events in the entire DM cohort in multiple prior clinical studies.

Long-Term Association of Brachial Artery Flow-Mediated Vasodilation and Cardiovascular Events in Middle-Aged Subjects with No Apparent Heart Disease

Michael Shechter [1], Assaf Issachar [1], Ibrahim Marai [1], Nira Koren- Morag [2], Yael Shahar [1], Alon Shechter [1], Micha Feinberg [1]

[1] Heart Institute, Chaim Sheba Medical Center and the Sackler Faculty of Medicine, Tel Aviv University, Tel Hashomer, Ramat Gan, [2] The Sackler Faculty of Medicine, Tel Aviv University, Tel Aviv, Israel

Background: Endothelial dysfunction is considered an important prognostic factor in atherosclerosis . The aim of this study was to find out the long-term association of peripheral vascular endothelial function and clinical outcome in healthy subjects with no apparent coronary artery disease (CAD).

Methods and Results: We prospectively assessed flow-mediated dilation (FMD) in 435 consecutive healthy subjects: 281 (65%) men, mean age 54 ± 12 years and body mass index 28 ± 4 kg/m^2. After overnight fasting and discontinuation of all medications for $\geq$ 12 hours, percent improvement in endothelium-dependent brachial artery FMD (%FMD) and endothelium-independent nitroglycerin (%NTG)-mediated vasodilatation were assessed using high resolution (15 MHz) linear array ultrasound. Subjects were divided into 2 groups: below (n=221) and above (n=214) the median %FMD of 10.7. The 2 groups were comparable in regard to CAD risk factors, lipoproteins, fasting glucose, hs-CRP, and concomitant medications. Subjects underwent clinical follow-up for a mean of 25 ± 2 months. The composite cardiovascular endpoints (all-cause mortality, non-fatal myocardial infarction, hospitalization for heart failure or angina pectoris, stroke, coronary artery bypass grafting and percutaneus coronary interventions) were significantly more common in subjects with %FMD below rather than above the median of 10.7% (11.8% vs 4.7%, p=0.007, respectively). Univariate analysis demonstrated that the median %FMD significantly predicted cardiovascular events [odds ratio (OR) of 2.78 and 95% CI (1.35 to 5.71) (p=0.003)]. After multivariate analysis that included conventional CAD risk factors, median %FMD was the best independent predictor of long-term cardiovascular adverse events [OR of 2.70 and 95% CI (1.16 to 6.32) (p=0.011)] (Figure).

Conclusions: Brachial artery median %FMD independently predicts long-term adverse cardiovascular events in healthy subjects in addition to those derived from traditional risk factor assessment.
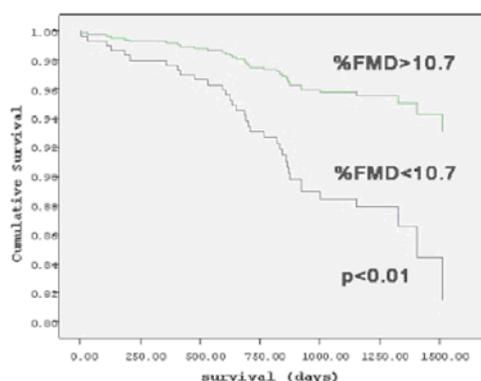
How Low Should HbA1c Levels be in Patients with Coronary Artery Disease?

Yaron Arbel [1], Noa Mashav [1], Shlomo Berliner [1], Itzhak Shapira [1], Itzhak Herz [2], Jacob George [2], Amir Halkin [2], Gad Keren [2], Ariel Finkelstein [2], Shmuel Banai [2]

[1] Pnimit, D, [2] Cardiology, Tel Aviv Medical Center, Tel Aviv, Israel

Introduction

Borderline fasting glucose has been implicated as a risk factor for Coronary artery disease (CAD). HbA1c is a biomarker of glucose control. We examined the potential link between HbA1c and the number of diseased vessels in a non-diabetic cohort.

Methods

We have prospectively collected patients undergoing angiography at the Tel Aviv Sourasky medical center. We included only patients that were non diabetic according to their medical history, were not taking any anti-diabetic medication and had an HbA1c$\leq$ 6mg%. A blood sample for HbA1c was taken during the angiography procedure. All patients gave their informed consent.

Results

We have collected 270 patients undergoing angiography. The correlation between the severity of CAD and HbA1c was 0.16 (p=0.01). We divided our population into 3 groups according to the extent of their CAD (1, 2, or 3 vessel disease). There was no difference in glucose levels between the groups. However, there was a significant difference in mean values of HbA1c between the groups (p=0.038). Patients with higher levels of HbA1c had a more extensive CAD (see below).
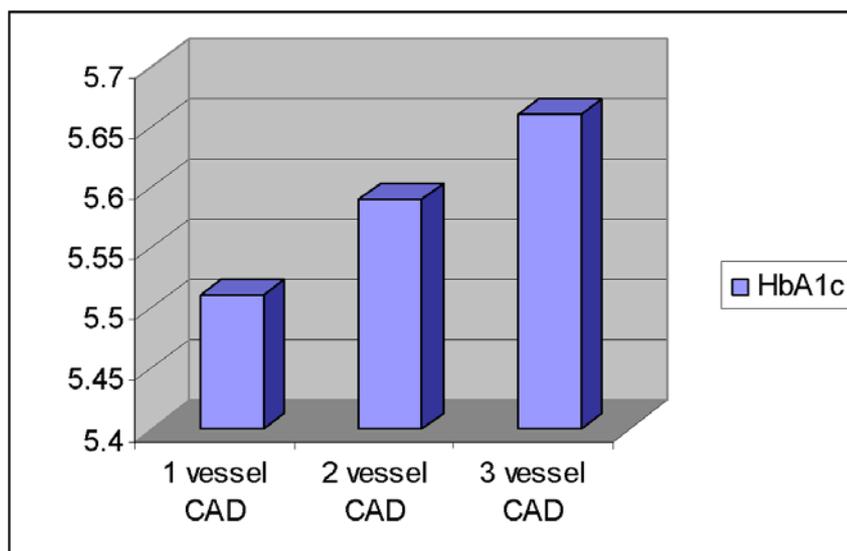
Conclusion

HbA1c at levels below than 6mg% may correlate to metabolic changes that result in CAD.

Cardiovascular Event Reduction in Diabetic Patients - Pharmacogenomic Application of the Haptoglobin Genotype

Uzi Milman[1], Chen Shapira[2], Shany Blum[3], Andrew P Levy[3]

[1] *Director, Clinical Research Unit, Clalit Health Services, Haifa and Western Galilee District,* [2] *Director, Carmel Medical Center,* [3] *Vascular Laboratory, Technion Faculty of Medicine, Technion-Israel Institute of Technology, Haifa, Israel*

Objective: Clinical trials of vitamin E have failed to demonstrate a decrease in cardiovascular events. However, these studies did not address possible benefit to subgroups with increased oxidative stress. Haptoglobin (Hp), a major anti-oxidant protein, is a determinant of cardiovascular events in patients with diabetes mellitus (DM). The Hp gene is polymorphic with two common alleles, 1 and 2. The Hp 2 allelic protein product provides inferior anti-oxidant protection compared to the Hp 1 allelic product. In retrospective analysis of HOPE DM participants with the Hp 2-2 genotype, vitamin E significantly reduced the incidence of myocardial infarction and cardiovascular death. We sought to validate this observation in a prospective trial. Additionally, a preplanned secondary analysis was to assess vitamin E influence on outcomes in those ICARE participants who were taking statins.

Methods and Results: 1434 DM individuals with the Hp 2-2 genotype were randomized to either vitamin E or placebo. The primary composite outcome was myocardial infarction, stroke and cardiovascular death. At the first evaluation of events, 18 months after initiating the study, the primary outcome was significantly reduced in individuals receiving vitamin E (2.2%) compared to individuals receiving placebo (4.7%) (p=0.01) and led to early termination of the study. Dual treatment with statins and vitamin E dramatically reduced the event rate compared to statin treatment alone. (1.3% (5/386) for vitamin E vs. 4.1% (17/415) for placebo

Conclusions: Vitamin E supplementation reduces cardiovascular events in individuals with DM and the Hp 2-2 genotype and augments statins reduction of cardiovascular events.

Lipid Levels among the African and Middle-Eastern Bedouin Populations

Jacob Dreiher [1,2,3], Arnon Cohen [1,3], Shimon Weitzman [2], Amir Sharf [1], Pesach Shvartzman [1,3]

[1] *Southern District, Clalit Health Services, Beer Sheva,* [2] *Department of Epidemiology,* [3] *Siaal Research Center, Department of Family Medicine, Ben Gurion University of the Negev, Beer Sheva, Israel*

Background: Previous studies observed higher high-density lipoprotein (HDL) levels and lower triglycerides levels among people of African ancestry. The goal of this study was to characterize lipid levels in Bedouins of African vs. Middle-Eastern ethnicity.

Methods: A cross-sectional study was conducted in a Bedouin primary care clinic in southern Israel, with 4470 listed individuals over the age of 21, of whom 402 (9%) were of African origin. A stratified random sample was included in the analysis. Associations between ethnicity, age, gender and lipid levels were assessed. Multiple linear regression and logistic regression models were used for multivariate analysis.

Results: The study included 261 African Bedouins and 406 Middle-Eastern Bedouins. (median age: 37 years, 58.6% females). The average total cholesterol and low-density lipoprotein (LDL) levels were 10 mg/dl lower among African Bedouins as compared to Middle-Eastern Bedouins (total cholesterol: 168.6 vs. 179.6 mg/dl, $p < 0.001$; LDL: 99.5 vs. 109.0 mg/dl, respectively , $p < 0.001$). Average triglycerides levels were 36 mg/dl lower among African Bedouins as compared to Middle-Eastern Bedouins (102.8 vs. 138.9 mg/dl, respectively, $p < 0.001$). Average HDL levels were 3 mg/dl higher among African Bedouins as compared to Middle-Eastern Bedouins (48.3 vs. 44.6 mg/dl, respectively, $p < 0.001$).

Conclusion: In conclusion, a lower prevalence of dyslipidemia was found in African Bedouins, as compared with Middle-Eastern Bedouins.