Cardiovascular Diseases in Patients with High Levels of Plasma High Density Lipoprotein: Association with Increased Plasma Oxidative State

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Abstract

Background: Increased levels of high density lipoprotein (over 60 mg/dl) are considered to be a negative risk factor for ischemic heart disease. However, some patients with high HDL still develop cardiovascular diseases.

Objective: To explore why patients with very high HDL still suffer from cardiovascular diseases.

Methods: We analyzed several risk factors, such as increased lipid peroxidation, hyperhomocysteinemia and increased release of inflammatory molecules, that could be related to the development of vascular disease in patients with high serum HDL levels. Patients with HDL cholesterol levels above 75 mg/dl were selected for this study and were separated into two groups based on the presence of atherosclerotic vascular disease, i.e., those with vascular disease (patients) and those without (controls).

Results: Plasma isolated from the patient group exhibited significantly increased lipid peroxidation by 21% and decreased total antioxidant status by 17%, but there were no differences regarding their serum or their paraoxonase activity. Moreover, both groups exhibited similar levels of serum C-reactive protein, fibrinogen and homocysteine, enabling us to eliminate these risk factors in the etiology of cardiovascular disease in the patient group.

Conclusion: Increased oxidative stress could be one of the factors leading to cardiovascular diseases in patients with high serum HDL levels.

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Epidemiologic studies have shown that elevated serum high density lipoprotein, over 60 mg/dl, is a negative risk factor for ischemic heart disease [1–3]. However some patients with high HDL develop coronary heart disease [4]. The heart estrogen/progestin replacement study (HERS) included only women with coronary heart disease, yet surprisingly, 20% had HDL cholesterol levels above 60 mg/dl [5]. Recent data suggest that certain risk factors can attenuate high HDL protection against CHD. For example, large HDL size is associated with a decreased risk of CHD, while small HDL may increase risk [6]. It was also shown recently that hypertension eliminates high HDL protection against CHD and stroke [7]. Oxidation and inflammation are both important processes involved in the development of atherosclerosis [8,9]. Oxidative stress is involved in the pathogenesis of atherosclerosis and is associated with lipid peroxidation of low density lipoprotein [10]. Serum paraoxonase, an HDL-associated esterase, was shown to protect against oxidative stress in lipoproteins and in atherosclerotic lesions, and this may be related to its hydrolytic properties against specific lipid peroxides [11,12]. PON1 was also shown to be inactivated by lipid peroxides [13]. Hyperhomocysteinemia has also been associated with increased risk of CHD and has been defined as an independent risk factor [14]. C-reactive protein and fibrinogen are both acute-phase proteins released during inflammatory response, and a rise in both serum CRP and fibrinogen levels is associated with increased risk for myocardial infarction [8].

In this study we analyzed several risk factors – increased lipid peroxidation, hyperhomeocysteinemia and increased release of inflammatory molecules – that could be related to the development of vascular disease in patients with high serum HDL concentrations.

Subjects and Methods

Patient population

Candidates with serum HDL cholesterol levels above 75 mg/dl and triglyceride levels under 200 mg/dl were selected for this study. Those with diabetes or cancer were not included. Based on the presence of atherosclerotic vascular disease, we separated these patients into two groups: those with vascular disease (patient group, n=12), and those without (control group, n=10). The patient group consisted of those who had suffered from myocardial infarction, angina pectoris, peripheral vascular disease, as well as patients after aorta-coronary bypass grafting or carotid endarterectomy.

Plasma oxidative stress determination

- *Plasma lipid peroxidation*. Human plasma was diluted (x 4) with phosphate-buffered saline and incubated in the absence or presence of 100 mmol/L of the free radical generator, 2,2'-Azobis 2-amidinopropane hydrochloride (Wako Chemical Industries Ltd, Japan) for 2 hours at 37°C. AAPH is a water-soluble azo compound that thermally decomposes to produce peroxyl radicals at a constant rate. Plasma lipid peroxidation was determined by measuring the lipid peroxides [15].

HDL = high density lipoprotein
CHD = coronary heart disease

PON1 = paraoxonase
CRP = C-reactive protein
AAPH = 2,2'-Azobis 2-amidinopropane hydrochloride

- *Total plasma antioxidant status*. Total antioxidant status was measured in plasma by a commercially available kit (Randox Laboratories Ltd, UK, Cat No. NX 2332) applicable for COBAS MIRA. Plasma was incubated with ABTS (2,2'-Azino-di-[3-ethylbenzthiazoline sulphonate]), a peroxidase (metmyoglobin) and H_2O_2, to produce a radical cation. The resulting product has a relatively stable blue-green color and is measured at 600 nm. Antioxidants in the added sample cause suppression of this color production to a degree that is proportional to their concentration [16].

Serum paraoxonase 1 activity

Serum paraoxonase activity was determined by its arylesterase activity using phenylacetate as the substrate. Initial rates of hydrolysis were determined spectrophotometrically at 270 nm. The assay mixture included 5 µl of serum, 1.0 mmol/L phenylacetate, and 0.9 mmol/L $CaCl_2$ in 20 mmol/L Tris HCl, pH 8.0. Non-enzymatic hydrolysis of phenylacetate was subtracted from the total rate of hydrolysis. The E_{270} for the reaction was 1,310 (mol/L) cm. One unit of arylesterase activity is equal to 1 mol of phenylacetate hydrolyzed/min/ml [11].

Serum determinations

Serum cholesterol and triglyceride concentrations were determined using commercial kits (Sigma, St Louis, USA). Serum C-reactive protein was measured by an immunoturbidimetric method [17]. Serum fibrinogen was measured by the Clauss method, using the Dade Thrombin Reagent [18]. Serum homocysteine was measured by high power liquid chromatography [19].

Statistical analysis

Student's paired *t*-test (two-tailed) was performed to compare the two groups (patients versus controls). For statistical analysis of parameters without Gaussian distribution such as triglycerides or plasma oxidizability, the values were transformed to logarithm values to create a Gaussian distribution. Results are given as mean ± standard error of the mean. A *P* value is indicated in Figure 1 and Table 1.

Results

The clinical characteristics, plasma lipid profiles and biochemical analysis of the patients and controls are shown in Table 1. There were no significant changes in the clinical characteristics of either group. Both groups had similar family history of heart disease, as well as similar prevalence of hypertension and smoking habits.

Plasma HDL cholesterol concentrations in both groups were over 75 mg/ml (77 ± 12 and 89 ± 18 mg/dl in the patient and the control group, respectively). Moreover, the plasma total cholesterol, as well as LDL cholesterol concentrations were slightly lower in the patient group. Overall, the ratio of plasma TC/HDL cholesterol in both groups was almost identical [Table 1].

LDL = low density lipoprotein
TC = total cholesterol

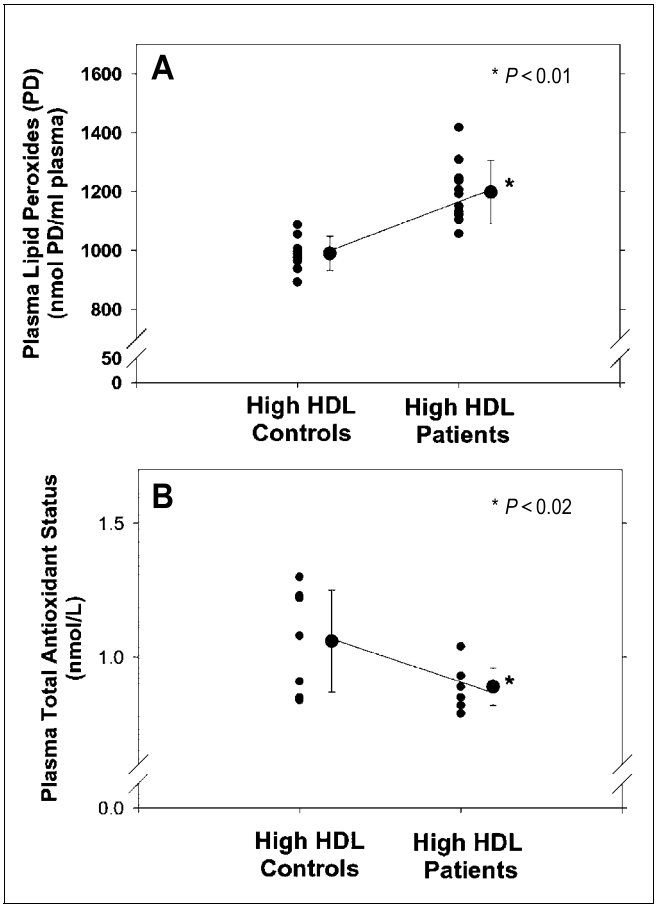

Figure 1. Plasma oxidative states in the patient and control group. **[A]** Susceptibility of plasma to AAPH-induced lipid peroxidation: plasma samples were diluted (x 4) in phosphate-buffered saline and lipid peroxidation was induced by plasma incubation with 100 mmol/L of AAPH for 2 hours at 37°C. The extent of plasma lipid peroxidation was determined by the lipid peroxide assay. **[B]** Plasma total antioxidant status was measured by a commercially available kit. Results are presented as individual measurements as well as mean ± SD.

We then analyzed possible risk factors that could distinguish between the patients and the controls. Inflammatory markers, which are associated with increased incidence of coronary heart disease, were determined. These markers include serum CRP and serum fibrinogen. There were no significant differences in these parameters or in serum homocysteine levels between the patient and the control group [Table 1].

We next analyzed the oxidative stress in both groups. First, we compared the plasma susceptibility to undergo lipid peroxidation in both groups. For this purpose, plasma isolated from both groups was incubated with AAPH and the plasma lipid peroxide content was determined. There was a significant increase by 21% ($P < 0.01$) in the susceptibility to oxidation in plasma isolated from the patient group as compared to the control group [Figure 1A]. To further assess whether the patient group was subjected to increased oxidative stress we analyzed the plasma total antioxidant status. As seen in Figure 1B, the total antioxidant status was significantly lower (by 17%, $P < 0.02$) in the patient group as compared to the control group.

Table 1. Clinical characteristics, lipid profile, inflammatory markers and homocysteine levels in the patient and control groups

Parameters	Controls (n=10)	Patients (n=12)
Age (yrs)	60 ± 10	66 ± 6
Smoking habits (%)		
Smoking today	20	8
Smoked in the past	20	25
Never smoked	60	67
Family history of heart disease (%)	40	47
Hypertension (%)	50	58
Glucose (mg/dl)	82 ± 4	80 ± 5
Total cholesterol (mg/dl)	279 ± 37	237 ± 30 *
HDL cholesterol (mg/dl)	89.3 ± 18	77 ± 11.9
LDL cholesterol (mg/dl)	173 ± 33	134 ± 47
Triglycerides (mg/dl)	84 ± 30	92 ± 28
TC/HDL cholesterol	3.1 ± 0.5	3.2 ± 0.5
C-reactive protein (mg/l)	3.8 ± 4.2	3.6 ± 2.8
Fibrinogen (mg/dl)	323 ± 66	338 ± 80
Homocysteine (mol/L)	9.1 ± 3.7	11.1 ± 7.9

* $P < 0.01$

Since serum paraoxonase 1 activity is inversely related to oxidative stress, we measured and compared this activity in serum from both groups by measuring its arylesterase activity. No significant differences in serum PON1 activity were found: 70.5 ± 4.1 U and 70.3 ± 6.7 arylesterase/ml in the patient and control groups respectively.

Discussion

Serum HDL exerts various potentially anti-atherogenic properties [2,3,20,21]. HDL is involved in reverse cholesterol transport, which involves efflux of cholesterol from non-hepatic cells, such as the arterial wall cells, and its subsequent delivery to hepatic cells and stereogenic organs [22].

Numerous clinical and epidemiologic studies have demonstrated the inverse correlation between serum HDL cholesterol levels and the risk of coronary heart disease [1–3]. However, the strength of association between HDL cholesterol and cardiovascular risk depends on the presence of additional risk factors. A certain percentage of the population with high serum HDL cholesterol exhibits events of CHD, meaning that specific additional risk factors can eliminate the protective effect of high HDL cholesterol over the risk of CHD [23]. The total cholesterol to HDL ratio has been cited as the strongest predictor of cardiovascular risk, with a ratio of <3.5 considered protective [24]. All the subjects in our study (both patients and controls) had a ratio lower than 3.5. The genetic background of cardiovascular diseases of the patients and the controls groups was almost identical.

The serum levels of CRP and fibrinogen, both markers of inflammatory processes, were identical in the two groups. Thus, inflammatory response does not seem to be the reason for the cardiovascular diseases in the patient group.

Serum PON1 activity was not different in the two groups, whereas oxidative stress was significantly increased in the patient group as compared to the control group. This was shown by an increased susceptibility to oxidation as well as a reduced total antioxidant status in the plasma of the patient group as compared to that in the control group. Oxidative stress has been shown to affect serum LDL lipids, as well as cellular lipids including those in arterial macrophages [9,10]. Oxidized LDL is taken up by arterial macrophages at an enhanced rate via scavenger receptors, leading to the formation of lipid-laden foam cells and accelerated atherosclerosis [10]. Thus, we suggest that the increased oxidative state in the patient group serum may be the underlying mechanism that makes this group more prone to develop cardiovascular diseases than the control group, with similar serum HDL levels. Moreover, oxidative stress also affected serum HDL in the patient group; and it has been shown that oxidized HDL is less efficient than native HDL in causing cholesterol efflux from arterial cells [25]. Therefore, although the patients had high serum HDL levels, their HDL could have been qualitatively damaged (oxidized), which might explain the inhibitory effect of oxidative stress on the protective effect of high serum HDL levels against the risk of vascular disease.

In this study we demonstrated that increased oxidative stress could be one of the factors leading to cardiovascular diseases in patients with high serum HDL concentrations.

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