Impact of Cigarette Smoking Cessation on High-Density Lipoprotein Functionality

– VN-SEESAW-HDL –

Kohei Takata, MD; Satoshi Imaizumi, MD, PhD; Emi Kawachi, PhD; Yasunori Suematsu, MD; Tomohiko Shimizu, MD, PhD; Satomi Abe, BSc; Yoshino Matsuo, PhD; Hitomi Tsukahara, PhD; Keita Noda, MD, PhD; Eiji Yahiro, MD, PhD; Bo Zhang, PhD; Yoshinari Uehara, MD, PhD; Shin-ichiro Miura, MD, PhD; Keijiro Saku, MD, PhD

Background: Smoking cessation reduces the risk of cardiovascular disease (CVD) and improves clinical outcomes in public health. We studied the effect of smoking cessation on high-density lipoprotein (HDL) functionality.

Methods and Results: We randomly treated 32 smokers with varenicline or a transdermal nicotine patch as part of a 12-week smoking cessation program (The VN-SEESAW Study). The plasma lipid profiles, plasma and HDL malondialdehyde (MDA) levels, HDL subfractions as analyzed by capillary isotachopheresis, cholesterol efflux capacity, and antiinflammatory activity of HDL were measured before and after the anti-smoking intervention. After smoking cessation, HDL-C, apoA-I levels and HDL subfractions were not significantly different from the respective baseline values. However, cholesterol efflux capacity and the HDL inflammatory index (HII) were significantly improved after smoking cessation. The changes in both parameters (% Δ cholesterol efflux capacity and Δ HII) were also significantly improved in the successful smoking cessation group compared with the unsuccessful group. The changes in cholesterol efflux capacity and HII also correlated with those in end-expiratory CO concentration and MDA in HDL, respectively.

Conclusions: Our findings indicate that smoking cessation leads to improved HDL functionality, increased cholesterol efflux capacity and decreased HII, without changing HDL-C or apoA-I levels or HDL subfractions. This may be one of the mechanisms by which smoking cessation improves the risk of CVD. (*Circ J* 2014; **78**: 2955–2962)

Key Words: Cholesterol efflux capacity; High-density lipoprotein (HDL); HDL inflammatory index; Malondialdehyde; Oxidative stress

Various epidemiological studies have revealed that cigarette smoking is positively correlated with the morbidity of cardiovascular diseases (CVD).^{1–5} Cigarette smoking has been recognized as a major cardiovascular risk factor and although the mechanisms by which cigarette smoking promotes arteriosclerotic lesions are not completely understood, they likely include changes in lipoproteins.

With regard to the lipid profile, it is well known that cigarette smoking increases plasma levels of low-density lipoprotein cholesterol (LDL-C), total cholesterol (TC) and triglycerides (TG), but decreases high-density lipoprotein cholesterol (HDL-C).^{6,7} Although a meta-analysis reported that the level of HDL-C significantly increased after smoking cessation, there were no

significant changes in LDL-C, TC, or TG.⁸ In another study, the activity of lecithin cholesterol acyltransferase (LCAT) was significantly decreased in smokers.⁹ LCAT activity is essential for the maturation of HDL, and affects HDL function.

HDL has several atheroprotective effects.¹⁰ A frequently discussed effect is that HDL promotes the transport of excess cholesterol from macrophage cells in peripheral tissues to the liver (ie, cholesterol efflux capacity). In addition to efflux capacity, HDL has antioxidant, antiinflammatory, antithrombotic or fibrinolytic¹¹ activities, and improves endothelial function by activating endothelial nitric oxide synthase.

A recent study demonstrated that the efflux capacity of HDL is negatively correlated with both carotid intima-media thick-

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Department of Cardiology (K.T., S.I., E.K., Y.S., T.S., S.A., Y.M., K.N., E.Y., Y.U., S.M., K.S.), Department of Biochemistry (B.Z.), Fukuoka University School of Medicine, Fukuoka; International University of Health and Welfare Fukuoka, Fukuoka (H.T.), Japan

Mailing address: Satoshi Imaizumi, MD, PhD or Keijiro Saku, MD, PhD, FACP, FACC, Department of Cardiology, Fukuoka University School of Medicine, 7-45-1 Nanakuma, Jonan-ku, Fukuoka 814-0180, Japan. E-mail: simaizumi@fukuoka-u.ac.jp or saku-k@fukuoka-u.ac.jp

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ness and coronary artery disease, independent of the HDL-C level.¹² It has also been shown that HDL from patients with endstage renal disease has impaired atheroprotective functions.¹³ The HDL inflammatory index (HII), which reflects the ability of HDL to prevent the oxidation of LDL/phospholipids, in patients with acute coronary syndrome was higher (less anti-oxidative) than in control subjects and patients with chronic coronary artery disease.^{14,15} Furthermore, it has been reported that the antiinflammatory and antioxidative capacities of HDL were impaired in patients with diabetes mellitus type 2.¹⁶ It is well known that HDL may be modified and lose its atheroprotective effects under chronic inflammation (ie, it can exhibit functional or dysfunctional properties).^{17–19} It has been reported that markers of functional HDL are apolipoprotein A-I (apoA-I) and paraoxonase 1, and those of dysfunctional HDL are apoC-III, serum amyloid A1 and lipoprotein-associated phospholipase A2 (Lp-PLA2).²⁰ Moreover, the modification of HDL by malondialdehyde (MDA), which is a product of lipid peroxidation, leads to impairment of its atheroprotective effects.^{21,22} Thus, HDL can become dysfunctional under certain conditions.

Cigarette smoking produces an environment similar to that of oxidative stress. However, little is known about the association between cigarette smoking and HDL function. In this study, we hypothesized that smoking cessation could lead to improved HDL function, and therefore we evaluated the cholesterol efflux capacity and HII in participants before and after smoking cessation.

Methods

Study Design

Tsukahara et al has been reported previously.²³ In short, 32 Japanese adult smokers aged 27–64 years were enrolled for treatment at the Smoking Cessation Clinic of Fukuoka University Hospital. Participants either received varenicline for 12 weeks or wore a transdermal nicotine patch on the chest for 8 weeks. Successful smoking cessation was identified by both a self-assessment report and the end-expiratory carbon monoxide (CO) concentration (<8 ppm).²³ The study protocol was approved by the Independent Review Board (IRB) of Fukuoka University Hospital (no. 7-05(08-27)), and all participants gave informed consent. The primary endpoint of the study was the incidence of smoking cessation in both groups at weeks 9–12 and weeks 9–24, and safety and the withdrawal symptoms at week 12. In the current investigation (VN-SEESAW-HDL), we tested plasma lipid profiles, plasma and HDL MDA levels, HDL subfractions as analyzed by capillary isotachopheresis (cITP), cholesterol efflux capacity, and the HII in plasma samples obtained at baseline (0 weeks) and 12 weeks in both the varenicline and nicotine patch groups, and considered the associated clinical data (including end-expiratory CO) collected from all the participants in this study. Although a plasma lipoprotein profile assay was approved by the IRB, the lipid peroxidation assay and measurements of cholesterol efflux capacity and HII were very new technologies, so the study protocol for VN-SEESAW-HDL was approved by the IRB at Fukuoka University Hospital (no. 14-5-13).

Assessment of Plasma Lipid Profiles and ApoA-I

Blood samples were collected after an overnight fast in 2, 2', 2'', 2'''-(ethane-1,2-diylidinitrilo) tetraacetic acid (EDTA)-containing tubes and centrifuged at 4°C and 3,000 rpm for 20 min. To prevent oxidation, collected plasma was frozen with 5 mmol/L EDTA, 0.2 mmol/L butylated hydroxytoluene and liquid nitrogen immediately after separation and preserved at

–80°C under N₂ gas.

Plasma levels of TG, LDL-C, and HDL-C were measured by enzymatic methods at the Department of Laboratory Medicine, Fukuoka University Hospital. Plasma apoA-I concentrations were measured using a commercially available enzymatic kit (ApoA-I Auto-N' Daiichi', Sekisui Medical Co Ltd, Tokyo, Japan).

Quantification of HDL Subfractions by cITP

Lipoprotein subfractions in plasma were quantified by cITP using a Beckman P/ACE MDQ system (Beckman-Coulter, Inc, Tokyo, Japan) as described previously.²⁴ cITP divides plasma lipoprotein into subfractions according to their electrophoretic charge. Plasma lipoprotein can be divided into 3 HDL subfractions: fast (f)-, intermediate (i)-, and slow (s)-migrating HDL. Levels of cITP-measured lipoprotein subfractions are expressed as the peak area relative to that of the internal marker. The 3 HDL subfractions can be characterized by their components. fHDL is composed of α -HDL and is rich in esterified cholesterol, whereas sHDL is composed of α - and pre β -HDL, and is lipid-poor and rich in apoE, apoA-IV, apoD, and apoJ.²⁵

Quantification of Plasma MDA and MDA in HDL

MDA results from lipid peroxidation, which is used as an indicator of oxidative stress in cells and tissues.²⁶ Cigarette smoke extract contains many oxidants, including free radicals, and is a source of oxidative stress. Therefore, we measured plasma MDA and MDA in HDL to evaluate the effects of smoking cessation, using a commercial kit to quantify the peroxidation of lipids (TBARS assay kit, Cayman Chemical Co Ltd, MI, USA) according to the manufacturer's instructions. In this assay, we used apolipoprotein B (apoB)-depleted plasma as HDL after precipitation of the apoB-containing lipoproteins by mixing plasma with phosphotungstate acid/MgCl₂ (Wako Pure Chemical Industries Co Ltd, Osaka, Japan).

Quantification of HDL-PAF-AH

Platelet-activating factor acetylhydrolase (PAF-AH) is a calcium-independent glycoprotein that belongs to the phospholipase A2 superfamily. In humans, circulating PAF-AH is mainly bound to LDL (80%), and a smaller amount is bound to HDL (20%). The mass of PAF-AH associated with HDL (HDL-PAF-AH mass) has been shown to have an antiatherogenic effect by inactivating LDL-derived oxidized phospholipids.²⁷ We measured the PAF-AH mass in apoB-depleted plasma (HDL-PAF-AH mass) to evaluate the effects of cigarette smoking. HDL-PAF-AH mass was measured using a commercial kit (Human PLA2G7/PAF-AH/Lp-PLA2 Immunoassay, R&D Systems, MN, USA) according to the manufacturer's instructions.

Assessment of Cholesterol Efflux Capacity

We examined the HDL cholesterol efflux capacity with an ex vivo system that used J774 macrophages and apoB-depleted plasma from the study participants as described previously¹² with some modifications. Briefly, J774 macrophages in 24-well plates were plated, cultured and radiolabeled with 2 μ Ci/ml of ³H-cholesterol in Dulbecco's modified Eagle's medium (DMEM) for 24 h. The day after labeling, the cells were washed and incubated in DMEM with 8-Br-cAMP for the upregulation of ATP-binding cassette A1 (ABCA1) transporter. Efflux medium containing apoB-depleted plasma (equivalent to 2% plasma, V/V, in the medium) was added for 4 h. For every sample, radiolabeled cholesterol counts were measured for both the cell compartment and media. The cholesterol efflux activity (%)

Table 1. Characteristics of the Study Group of Japanese Smokers

	Overall (n=28)	Successful group (n=21)			Unsuccessful group (n=7)		
	Baseline	Baseline	After 12 weeks	P value	Baseline	After 12 weeks	P value
Age, years	46±12	46±12			46±12		
Male, n (%)	23 (82)	17 (81)			6 (86)		
BMI, kg/m ²	23.7±3.4	23.7±3.8	24.5±3.8	<0.001	23.4±2.0	24.1±2.7	0.079
PR, bpm	78±9	77±9	70±11	0.016	79±10	76±7	0.23
SBP, mmHg	129±18	130±19	124±15	0.039	125±14	130±16	0.33
DBP, mmHg	78±10	78±11	76±11	0.47	79±5	80±11	0.78
Hb, g/dl	15.0±1.2	15.0±1.2	14.5±1.1	<0.001	15.3±1.0	14.6±1.4	0.018
End-expiratory CO, ppm	27±11	23±10	4±2	<0.001	38±8	17±12	0.0021
Hypertension, n (%)	3 (11)	2 (10)			1 (14)		
Dyslipidemia, n (%)	2 (7)	1 (5)			1 (14)		
Diabetes mellitus, n (%)	0 (0)	0 (0)			0 (0)		
Hyperuricemia, n (%)	1 (4)	0 (0)			1 (14)		
Varenicline, n (%)	14 (50)	10 (48)			4 (57)		
ACEI/ARB, n (%)	1 (3)	1 (5)			0 (0)		
CCB, n (%)	3 (11)	2 (10)			1 (14)		
β-blocker, n (%)	1 (3)	1 (5)			0 (0)		
Statins, n (%)	2 (7)	1 (5)			1 (14)		

Values are mean±SD or %. Differences between values at baseline and after 12 weeks were evaluated with the paired t-test.

ACEI, angiotensin-converting enzyme inhibitor; ARB, angiotensin receptor blocker; BMI, body mass index; CCB, calcium-channel blocker; CO, carbon monoxide; DBP, diastolic blood pressure; Hb, hemoglobin; PR, pulse rate; SBP, systolic blood pressure.

was calculated by the following formula: (radioactivity in the medium/total radioactivity (radioactivity in medium+cells extracted with NaOH/NaCl))×100–cholesterol efflux activity in plasma-free medium.

Assessment of HII

The HII was determined by cell-free assay systems using 2',7'-dichlorofluorescein-diacetate (DCFH-DA) with modification of a previously published method using oxidized PAPC (Ox-PAPC) as the fluorescence-inducing agent.¹⁴ This assay is based on the ability of HDL to prevent the formation of or inactivate oxidized phospholipids.²⁸ The presence of Ox-PAPC leads to the conversion of normally nonfluorescent DCFH-DA into a fluorescent form of DCFH. After the precipitation of apoB-containing lipoproteins, HDL-containing supernatant was used in the assay. DCFH-DA (Invitrogen Applied Biosystems, Carlsbad, CA, USA) was first dissolved in fresh methanol at 2.0 mg/ml and then incubated in the dark at room temperature for 20 min to release DCFH. Ox-PAPC was prepared from PAPC (Avanti Polar Lipids, Alabaster, AL, USA) as previously described.²⁸ Next, 25 μl of Ox-PAPC (0.2 mg/ml) and a fixed volume of apoB-depleted plasma (5 μl) from the study subjects were incubated at 37°C with phosphate-buffered saline in black, flat-bottom microtiter plates (BD Falcon) for 30 min with rotation. We then added 25 μl of DCFH solution (0.2 mg/ml) to each well and incubated at 37°C for 1 h with rotation. Fluorescence at an excitation wavelength of 485 nm and an emission wavelength of 535 nm was measured after incubation with a plate reader (TriStar LB941, Berthold Technologies). HII values were calculated by the following formula: fluorescence intensity in subject samples+Ox-PAPC/fluorescence intensity in Ox-PAPC alone.

Assessment of the Antioxidative Activity of HDL Using AAPH

HDL from the study participants and LDL from a normolipidemic donor were isolated by ultracentrifugation (HDL: 1.063<d<1.21, LDL: 1.019<d<1.063, respectively). LDL

(75.4 mg/dl cholesterol) was oxidized with 1 mmol/L 2,2'-azobis-(2-amidinopropane) hydrochloride (AAPH) for 24 h at 37°C with HDL (10.1 mg/dl cholesterol). HDL was added to LDL directly before oxidation. The degree of LDL oxidation was measured by a TBARS assay kit according to the manufacturer's instructions.^{29,30}

Statistical Analysis

We registered 32 smokers for comparison of HDL functions before and after smoking cessation. Data are presented as the mean±standard deviation (SD). Changes were defined as and calculated as: value after 12 weeks – value at baseline. Differences between values at baseline and after 12 weeks were evaluated with the paired t-test. The associations of HDL functions with other parameters were assessed in terms of Pearson's product-moment correlation coefficient or Spearman's rank correlation coefficient. Differences between the successful and unsuccessful smoking cessation groups were evaluated with the unpaired t-test. A P value of less than 0.05 was considered statistically significant. Analyses were performed using SAS software (version 9.3, SAS Institute, Cary, NC, USA).

Results

Characteristics of the Study Participants

Although 32 smokers were registered (16 for varenicline, 16 for a nicotine patch), 4 dropped out within 12 weeks. Of the remaining 28 participants who were analyzed, 7 did not stop smoking. The proportions of participants who did not successfully stop smoking were not significantly different between the varenicline (4 participants) and nicotine patch (3 participants) groups. At baseline, there were no statistically significant differences in plasma lipid profiles or HDL subfractions between the successful smoking cessation group and the unsuccessful group. Among all the participants who stopped smoking, there were statistically significant differences between baseline and after 12 weeks with respect to body mass index (BMI), pulse

	Overall (n=28)				Successful group (n=21)			Unsuccessful group (n=7)		
	Baseline	Baseline	After 12 weeks	P value	Baseline	After 12 weeks	P value	Baseline	After 12 weeks	P value
Triglyceride, mg/dl	251±284	198±146	192±196	0.83	410±501	393±346	0.94			
LDL-C, mg/dl	109±22	110±19	109±25	0.82	104±30	115±32	0.42			
HDL-C, mg/dl	55±14	57±14	57±14	0.50	49±14	44±9	0.15			
ApoA-I, mg/dl	116±23	116±21	121±13	0.22	112±31	112±17	0.95			
fHDL	1.58±0.49	1.68±0.49	1.66±0.52	0.88	1.29±0.36	1.18±0.21	0.20			
iHDL	2.36±0.39	2.43±0.39	2.47±0.65	0.79	2.13±0.30	2.03±0.27	0.41			
sHDL	0.33±0.10	0.33±0.08	0.38±0.13	0.15	0.32±0.14	0.39±0.22	0.13			

Values are mean ± SD. Differences between values at baseline and after 12 weeks were evaluated with the paired t-test. ApoA-I, apolipoprotein A-I; fHDL, fast-migrating HDL; HDL, high-density lipoprotein; HDL-C, HDL cholesterol; iHDL, intermediate-migrating HDL; LDL-C, low-density lipoprotein (LDL) cholesterol; sHDL, slow-migrating HDL.

rate (PR), systolic blood pressure (SBP), hemoglobin (Hb), and end-expiratory CO concentration (Table 1).

Lipid-Related Parameters and HDL Subfractions

In both the successful and unsuccessful smoking cessation groups, there were no statistically significant differences in the lipid profiles between baseline and after 12 weeks (Table 2). Various methods can be used to assess HDL subfractions, and the analysis of HDL subfractions may be more useful than the HDL-C level as a predictor of CVD.³¹ Several studies have

reported that cigarette smoking reduces the HDL2 fraction.³² In terms of the HDL subfractions as determined by cITP in our study, each subfraction (ie, fast-, intermediate-, and slow-migrating HDL) positively correlated with the HDL-C and apoA-I levels (data not shown). On the other hand, there were no statistically significant changes in the HDL subfractions between baseline and after 12 weeks in either the successful or unsuccessful groups (Table 2). Representative cITP data for a participant in the successful smoking cessation group showed no difference between before and after smoking cessation

Table 3. MDA-Related Parameters and HDL Functions in the Study Group of Japanese Smokers

	Overall (n=28)	Successful group (n=21)			Unsuccessful group (n=7)		
	Baseline	Baseline	After smoking cessation	P value	Baseline	After 12 weeks	P value
Plasma MDA, $\mu\text{mol/L}$	1.88 $\pm$ 1.00	1.73 $\pm$ 0.58	1.57 $\pm$ 0.50	0.03	2.35 $\pm$ 1.75	2.02 $\pm$ 0.43	0.63
MDA in HDL, $\mu\text{mol/L}$	0.55 $\pm$ 0.11	0.53 $\pm$ 0.08	0.47 $\pm$ 0.09	0.003	0.60 $\pm$ 0.17	0.50 $\pm$ 0.07	0.30
Cholesterol efflux capacity, %	13.89 $\pm$ 2.53	14.15 $\pm$ 2.46	14.83 $\pm$ 2.35	0.01	13.09 $\pm$ 2.74	12.56 $\pm$ 1.97	0.48
HII	1.15 $\pm$ 0.59	1.13 $\pm$ 0.31	0.98 $\pm$ 0.18	0.01	0.78 $\pm$ 0.20	1.00 $\pm$ 0.19	0.06
AAPH assay, * %	94.01 $\pm$ 15.90	91.57 $\pm$ 15.43	97.02 $\pm$ 16.48	0.15	100.66 $\pm$ 16.40	105.65 $\pm$ 5.33	0.51

Values are mean $\pm$ SD. Differences between values at baseline and after 12 weeks were evaluated with the paired t-test. *Values expressed as % of LDL alone, n=16 for Successful group and n=6 for Unsuccessful group.

HII, HDL inflammatory index; MDA, malondialdehyde. Other abbreviations as in Table 2.

Figure 2. Extent of the changes in high-density lipoprotein (HDL) function. (A) Differences in % Δ cholesterol efflux capacity between the successful and unsuccessful smoking cessation groups. (B) Differences in the Δ HDL inflammatory index (Δ HII) between the successful and unsuccessful smoking cessation groups. Changes are defined as Δ and calculated as value after 12 weeks – value at baseline. Differences between the groups were evaluated with the unpaired t-test.

(Figures 1A,B). Furthermore, there were also no statistically significant differences in the changes in HDL subfractions between the successful and unsuccessful smoking cessation groups (Figures 1C–E).

Plasma MDA and MDA in HDL

Both the plasma MDA and MDA in HDL levels decreased after successful smoking cessation, but did not significantly decrease after 12 weeks in the unsuccessful group (Table 3). Thus, smoking cessation reduced oxidative stress in HDL as well as plasma. In addition, there was a significant relationship between the amount of change in the plasma MDA level (Δ plasma MDA) (Δ =value after 12 weeks–value at baseline) and that in the MDA in HDL level (Δ MDA in HDL) ($r=0.50$, $P=0.007$).

HDL-PAF-AH

There were no significant differences in either HDL-PAF-AH mass (at baseline and after 12 weeks) or Δ HDL-PAF-AH between the successful and unsuccessful smoking cessation groups (data not shown).

Cholesterol Efflux Capacity of HDL

Cholesterol efflux capacity was significantly improved after 12 weeks in the successful smoking cessation group, but there was

no significant increase after 12 weeks in the unsuccessful group (Table 3). In addition, the %change in efflux capacity was improved in the successful group compared with the unsuccessful group (Figure 2A).

On the other hand, the % Δ cholesterol efflux capacity correlated with Δ end-expiratory CO ($r=-0.45$, $P=0.02$). There was no statistically significant difference between the varenicline and transdermal nicotine patch groups with regard to cholesterol efflux capacity (data not shown).

HII

In the successful smoking cessation group, HII was significantly improved after smoking cessation (Table 3) and Δ HII in the successful cessation group was improved compared with the unsuccessful group (Figure 2B). The correlation between HII and cholesterol efflux capacity, at both baseline and after 12 weeks, was insignificant (data not shown). In addition, there was no significant difference in the antioxidative activity of HDL analyzed by AAPH assay between the successful and unsuccessful smoking cessation groups (Table 3). Thus, these functions of HDL might reflect other properties of HDL. Further, there was no statistically significant difference in HII between the varenicline and transdermal nicotine patch groups (data not shown).

Table 4. Correlations Between HDL Functions and HDL-Related Parameters at Baseline in Study Group of Japanese Smokers

	Cholesterol efflux capacity (n=28)		HDL inflammatory index (n=28)	
	r	P value	r	P value
BMI	-0.44	0.02	0.25	0.20
HDL-C	0.53	0.004	0.016	0.94
ApoA-I	0.41	0.03	0.07	0.73
MDA in HDL	0.08	0.69	0.37	0.06

At baseline, the associations between HDL functions and other parameters were assessed in terms of Pearson's product-moment correlation coefficient or Spearman's rank correlation coefficient. Abbreviations as in Tables 1–3.

Correlations Between HDL Function and HDL-Related Parameters

In terms of the relationships at baseline, cholesterol efflux capacity was positively correlated with HDL-C and apoA-I levels, and negatively correlated with BMI (Table 4). HII at baseline was associated with the MDA in HDL level (Table 4) and AMDA in HDL was also associated with Δ HII ($r=0.43$, $P=0.02$).

Discussion

In our study, there were no changes in HDL-C or apoA-I levels, HDL-PAF-AH mass, or HDL subfractions after a 12-week smoking cessation program. Nevertheless, both cholesterol efflux capacity and HII improved. There was no significant association between the changes in %cholesterol efflux capacity and HII. Our findings indicate that cholesterol efflux capacity and HII might reflect other properties of HDL, and that the improvement in HDL function is independent of any changes in the levels of HDL-C, apoA-I and HDL subfractions. Furthermore, Δ CO and Δ MDA in HDL were significantly associated with cholesterol efflux capacity and HII, respectively. The end-expiratory CO concentration was associated with the number of cigarettes smoked.³³ In our study, the end-expiratory CO and MDA in HDL concentration both decreased after successful smoking cessation (Tables 1,4). Taken together, our findings further suggest that smoking cessation itself might be associated with improvement of HDL function through a reduction in the level of MDA in HDL.

Several recent studies have failed to find an association between an increase in the HDL-C level and the CVD risk.^{34,35} HDL is heterogeneous, and consists of various components that affect its properties. These components change according to the conditions, which can result in either functional or dysfunctional HDL. These changes result from loss or modification of proteins, lipids and enzymes. Thus, environmental changes are important factors in determining HDL properties. In particular, oxidative stress, which is brought about by cigarette smoking, can lead to functional impairment of HDL. Our findings also suggest that lipid peroxidation is significantly associated with HDL function. Moreover, our study showed that smoking cessation can improve HDL function within a very short time (ie, only 12 weeks). This finding may contribute to epidemiological data showing that the risk of CVD is reduced to less than half within 1 year of smoking cessation.³ This is the first study to demonstrate that both cholesterol efflux capacity and HII can improve within a short time after smoking cessation.

It has been reported that elevation of the HDL-C level, which is brought about by smoking cessation, reduces the risk of

CVD by 7.4 percentage points, to 9.0% in males and 12.5% in females.⁸ As a possible explanation for why the HDL-C level did not increase after smoking cessation in our study, the increased BMI after smoking cessation may have had an effect. In fact, BMI was inversely correlated with the HDL-C level at baseline (HDL-C: $r=-0.55$, $P=0.002$). The amount of change in HDL-C in the successful smoking cessation group was significantly greater than that in the unsuccessful group. In addition, Δ HDL-C was not significantly correlated with either % Δ cholesterol efflux capacity or Δ HII (data not shown). These data suggest that the amount of change in HDL-C was not associated with improved HDL functionality in our study.

It has been demonstrated that HDL-PAF-AH has antiatherogenic properties.³⁶ With regard to cigarette smoking, a previous study demonstrated that both the activity and mass of HDL-PAF-AH in current smokers were lower than in non-current smokers.³⁷ In contrast, another study reported that there was no significant difference in HDL-PAF-AH activity between current and non-current smokers.³⁸ Thus, the relationships between cigarette smoking and HDL-PAF-AH activity and mass are still controversial.

The level of pre β 1-HDL, which is a component of pre β -HDL and the primary acceptor of cholesterol effluxed by the ABCA1 transporter, is positively associated with efflux capacity in vitro.³⁹ Recently, several clinical trials have demonstrated that the level of pre β 1-HDL is increased in an atherogenic state, such as coronary artery disease.^{40–42} In our study, there were no differences in the HDL subfractions between baseline and after smoking cessation (Table 2). The 3 HDL subfractions as assessed by cITP can be characterized according to their components: fHDL is composed of α -HDL and is rich in esterified cholesterol, sHDL is composed of α - and pre β -HDL, and is lipid-poor and rich in apoE, apoA-IV, apoD, and apoJ.²⁵ It is possible that smoking cessation does not significantly change the HDL subfractions or the pre β 1-HDL level.

According to recent reports,⁴³ the quality of HDL may be more important than its quantity. In the present study, cholesterol efflux capacity and HII were improved compared with baseline, despite a lack of changes in the levels of HDL-C, apoA-I and HDL subfractions. Cholesterol efflux capacity and HII improved in proportion to decreases in end-expiratory CO and MDA in HDL, respectively, after smoking cessation. Furthermore, the choice of therapeutic agent did not influence the improvement in HDL function. Thus, smoking cessation itself, rather than any other effects of the therapeutic agent, is important for restoring the functionality of HDL. Our findings suggest that smoking cessation itself plays a key role in the atheroprotective effects of HDL, which is more important than raising the level of HDL-C. Lipid peroxidation is considered to be a cause of impaired HDL functionality.

With respect to the effect of cigarette smoking on HDL functionality, there have been several reports involving basic medical science. First, cigarette smoking is a major source of oxidative stress. Our data further support the finding that denatured HDL, which was incubated with whole cigarette smoke extract or copper ion, showed reduced efflux capacity in vitro.^{44,45} The formation of lipid peroxidation derivatives is linked to changes in the configuration and properties of HDL.⁴⁶ MDA modifies Lys residues in apoA-I, which leads to HDL dysfunction in vivo.²¹ The modification of HDL particles by oxidative stress is a major factor contributing to dysfunctional HDL. We can assume that other cigarette components also affect HDL functionality.

Cholesterol ester (CE) is taken up via scavenger receptor type B1 (SR-B1) receptors on hepatocytes, which recognize

apoA-I as a ligand, as a direct pathway of hepatic cholesterol uptake. In addition to attenuating efflux capacity in smokers, cigarette smoking impairs the hepatic uptake of HDL-C.⁴⁷ As a result of these interactions, the reverse cholesterol transport of HDL may be impaired.

It is well known that cigarette smoking is associated with endothelial dysfunction, which is restored by smoking cessation. Because HDL is closely related to endothelial function, improved HDL functionality, which is brought about by smoking cessation, may help to improve vascular endothelial function. Our findings raise the possibility that smoking cessation may reduce the risk of CVD through improved HDL functionality.

Study Limitations

The sample size was small, which limits generalization of the results. Furthermore, this study assessed HDL functionality only over the short term. The long-term effect of smoking cessation on HDL functionality will need to be elucidated in the future.

Conclusions

Our findings indicate that smoking cessation leads to improved HDL functionality, increased cholesterol efflux capacity and decreased HII, without changing the levels of HDL-C or apoA-I or HDL subfractions. Moreover, the level of MDA in HDL, which can modify apoA-I and impair its function, decreased after smoking cessation. This may be one of the mechanisms by which smoking cessation improves the risk of CVD.

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