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HDL lipid composition is profoundly altered in patients with type 2 Diabetes and Atherosclerotic Vascular Disease

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Abstract

Background and Aims—We have previously shown that the anti-inflammatory and anti-oxidant functions of HDL are impaired in T2D patients. In this study, we examined whether HDL from T2D patients contains elevated levels of oxidized fatty acids and whether those levels correlate with cardiovascular disease (CVD).

Methods and Results—HETEs and HODEs on HDL were determined by LC-MS/MS in 40 non-diabetic controls (ND), 40 T2D without CVD (D^+CVD^-) and 38 T2D with known history of CVD (D^+CVD^+). HDL oxidant index was evaluated by a cell-free assay using dichlorofluorescein. Twenty-six randomly selected subjects from the three groups underwent coronary calcium score evaluation (CAC).

Major cardiovascular risk factors were similar among the groups. HETEs and HODEs content were significantly increased in HDL from D^+CVD^+ when compared to D^+CVD^- and ND patients. HDL oxidant index was not different among the three groups; however, it was significantly higher in patients with CAC score >100 when compared to patients with CAC score <100.

Conclusion—Patients with D^+CVD^- and D^+CVD^+ are characterized by a severe, graded enrichment of oxidized fatty acids on HDL. In the present study, a loss of HDL function (as

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estimated by the HDL oxidant index) is observed only in patients with more advanced atherosclerosis.

Introduction

Recent evidence (1) suggests that the anti-oxidant activity of HDL is defective in patients with atherogenic dyslipidemia. The diabetic population has atherogenic dyslipidemia but also has low HDL-cholesterol levels, suggesting that the composition of HDL – as determined by the proteome and/or the lipidome – may be an important determinant of HDL dysfunction in this population. We have previously demonstrated that the anti-oxidant and anti-inflammatory properties of HDL are impaired in type 2 diabetes (T2D) patients compared to healthy control subjects (2).

Lipid composition plays an important role in maintaining normal HDL metabolism and function. HDL is an assembly of a neutral lipid core and an outer shell consisting of polar lipids and proteins with distinct chemical and physical properties including density flotation, protein composition, molecular size, and electrophoretic migration. Overall, the studies of proteomics and lipidomics of HDL clearly demonstrated that HDL is a complex heterogeneous mixture of particles (3). In humans, proteomic analysis demonstrated changes in HDL particles from a pro-atherogenic to a less pro-atherogenic composition during 6 months of rituximab treatment (4). Moreover, pro-inflammatory HDL in patients with rheumatoid arthritis contains a significantly altered proteome, including increased amounts of acute-phase proteins (5). Furthermore, an enrichment in smaller HDL particles was associated with vascular inflammation within the thoracic aorta in patients with psoriasis, suggesting that HDL characteristics play an important role in psoriatic vascular inflammation (6). Finally an increased abundance of oxidized methionine residues has been found on HDL isolated from type 2 diabetic patients compared to healthy controls (7).

One of the major roles of HDL is to protect LDL from free-radical-mediated oxidation. The anti-oxidant properties of apolipoprotein A-I (apoA-I) (8; 9) as well as other HDL components including paroxonase-1 (PON-1)(10) and platelet-activating factor-acetyl hydrolase (PAF-AH)(3; 11) a are implicated in the prevention of LDL oxidation. Most of the lipid peroxidation products, which have been implicated in both HDL and LDL modification and in the pathogenesis of atherosclerosis are derived from the degradation of the polyunsaturated omega-6 fatty acids, arachidonic acid (AA) and its precursor linoleic acid (LA). In response to a variety of stimuli including growth factors and cytokines, several phospholipases which act on membrane phospholipids can be activated and release AA (12; 13). Free AA can be converted to bioactive eicosanoids through the cyclooxygenase (COX) and lipoxygenase (LOX) enzymes catalyzing the formation of hydroperoxyeicosatetraenoic acids which lead to the formation of their final product: hydroeicosatetraenoic acids (HETEs)(12). Other species of monohydroxy fatty acids are the hydroxyoctadecadienoic acids (HODEs), which are generated enzymatically by COX and LOX and cytochrome P450 pathways directly from LA; however the bulk of linoleic acid peroxidation occurs by non-enzymatic processes. HODEs are excellent indicators of free-radical-mediated lipid peroxidation (14).

We have previously shown that in livers from streptozotocin-diabetic mice there was an increased concentration of AA and its peroxidation products (HETEs and HODEs), which were reduced by a long-term treatment with apoA-I mimetic peptides (15). Next, we demonstrated that HDLs from diabetic patients display a loss of anti-oxidant and anti-inflammatory function (2), and suggested that the lipoprotein content of oxidized fatty acids could play a role in this loss of function. In the present study we examined whether HDLs from T2D patients contain elevated levels of oxidized lipids and whether these changes relate to the degree of metabolic control and/or clinical evidence of atherosclerotic cardiovascular disease (CVD).

Methods

Study Population

Forty consecutive patients with type 2 diabetes without known cardiovascular disease (D^+CVD^-) and 38 consecutive patients with type 2 diabetes and known CVD (D^+CVD^+), between the age of 40 and 80 years attending the outpatient clinic of the department of Internal Medicine were recruited within a 12-month period. Forty age- and sex-matched non-diabetic patients (ND) without CVD were recruited both from the Internal Medicine outpatient clinic and from the clinic personnel.

After giving their written informed consent, participants returned (within 7 days) in fasting condition for blood sampling (approx. 80 ml), a visit, and an interview done by the same investigator. Exclusion criteria were BMI >40 kg/m^2, any acute or chronic inflammatory disease, any previous diagnosis of cancer, moderate-severe chronic kidney or liver disease, and regular or frequent use of anti inflammatory drugs or antioxidants. Type 2 diabetes is defined on the basis of the global guidelines for type 2 diabetes (16).

Known CVD was defined as previous (>6 months) acute coronary heart disease (including possible fatal or non-fatal MI, hospitalized unstable angina, resuscitated cardiac arrest not attributed to a non-CHD cause, coronary artery bypass graft or any other coronary revascularization) or previous cerebrovascular disease (including stroke and transient ischemic attack) or peripheral vascular disease (ankle-brachial index<0.9, claudication + pathological vascular arterial Doppler study, peripheral artery revascularization or amputation).

Biochemical measurements

Blood was collected in tubes for routine biochemistry and in 4x10 ml-EDTA tubes (40 ml) and immediately centrifuged; the plasma was stored in 1-ml aliquots at −80C° for subsequent analysis.

HDL anti-oxidant properties—HDL used for this experiment was isolated by the dextran sulfate method and freshly analyzed. Fifty microliters of HDL Magnetic Bead Reagent (Polymedco, Cortland Manor, NY) were mixed with 250 μl of subject's plasma and first incubated 5 minutes at room temperature then additional 5 minutes on a magnetic particle concentrator. HDL cholesterol in the supernatant was quantified using a standard assay (Thermo DMA, San Jose, CA).

The dichlorofluorescein (DCF) assay was performed as described previously (17) with slight modifications. Briefly, 25 μl of standard LDL (sLDL) solution containing 2.5 μg LDL-cholesterol and 25 μl HDL from each patient (2.5 μg of HDL-cholesterol) were incubated in 96 well plates for 30 min at 37°. Then 25 μl of H_2DCF-DA solution was added to each well, and after 60 min of incubation at 37°C fluorescence intensity was measured. Values for the fluorescence intensity of DCFH induced by test HDL+sLDL was normalized to sLDL alone in order to obtain an index. Dysfunctional HDL is unable to prevent the air oxidation of LDL that occurs spontaneously *in vitro*, and may actually increases oxidation thereby being classified pro-oxidant. Thus, index values equal to or greater than 1.0 indicate dysfunctional HDL, (pro-oxidant HDL) while values smaller than 1.0 indicates normal, anti-oxidant HDL. Values for intra- and interassay coefficient of variability of the DCF assay were 0.5 ± 0.37% and 3.0 ± 1.7%, respectively.

Determination of HDL lipid peroxidation products associated with HDL—HDL for this experiment was isolated by refrigerated sequential density ultracentrifugation (in the density range of 1.063–1.21 g/mL) from heparinized plasma maintained with the addition of 20μM BHT and 5mM EDTA throughout.

Liquid chromatography-tandem mass spectrometry (LC/ESI/MS-MS) was performed using a quadrupole mass spectrometer (4000 QTRAP; Applied Biosystems Division, Life Technologies, Carlsbad, CA) equipped with electrospray ionization (ESI) source. Chromatography was performed using a Luna C-18(2) column (3μm particle, 150 × 3.0 mm; Phenomenex) with a security guard cartridge (C-18; Phenomenex) at 40°C. Detection was accomplished by using the multiple reaction monitoring (MRM) mode with negative ion detection. Ultracentrifugally isolated HDL samples were then spiked with an internal standard mixture (15(S)-HETE-d_8, 12(S)-HETE-d_8, 5(S)-HETE-d_8, 13(S)-HODE-d_4, 10 ng/ml each) in methanol. The HDL/internal standard sample was then loaded onto a preconditioned Oasis HLB solid-phase extraction (SPE) cartridge (1 ml, 10 mg) on a vacuum manifold (Waters). The SPE cartridge was equilibrated with 1 ml methanol followed by 1 ml water before sample loading. After the sample was loaded, the cartridge was washed with 1 ml 5% methanol in water before free fatty acids were subsequently eluted in 1 ml of methanol. This eluate was evaporated under argon and reconstituted with 60 μl of methanol, vortexed and transferred to an auto sampler vial for LC/ESI/MS-MS analysis. LC/ESI/MS-MS analysis was performed as described previously (18). The results were adjusted to cholesterol levels in HDL. The transitions monitored were mass-to-charge ratio (m/z): 319.1→179.0 for 12-HETE; 319.1→219.0 for 15-HETE; 295.0→194.8 for 13-HODE; 319.1→115.0 for 5-HETE; 295.0→171.0 for 9-HODE; 327.1→226.1 for 15(S)-HETE-d_8; 327.1→184.0 for 12(S)-HETE-d_8; 299.0 →197.9 for 13(S)- HODE-d_4, 327.1→115.9 for 5(S)-HETE-d_8. *Chemicals*: (±)12-hydroxy-5Z,8Z,10E, 14Zeicosatetraenoic acid (12-HETE), (±)15-hydroxy-5Z,8Z,11Z,13E-eicosatetraenoic acid (15-HETE), (±)13-hydroxy-9Z,11E-octadecadienoic acid (13-HODE), (±)9-hydroxy-10E, 12Zoctadecadienoic acid (9-HODE), (±)5-hydroxy-6E,8Z,11Z,14Z-eicosatetraenoic acid (5-HETE),12(S)-hydroxy-5Z,8Z,10E,14Z-eicosatetraenoic-5,6,8,9,11,12,14,15-d_8 acid (12(S)-HETE-d_8),15(S)-hydroxy-5Z,8Z,11Z,13E-eicosatetraenoic-5,6,8,9,11,12,14,15-d_8 acid (15(S)- HETE-d_8), 5(S)-hydroxy-6E,8Z,11Z,14Z –eicosatetraenoic-5,6,8,9,11,12,14,15-d_8

acid (5(S)- HETE-d_8), 13(S)-hydroxy-9Z,11E-octadecadienoic-9,10,12,13-d_4 acid (13(S)-HODE-d_4), were purchased from Cayman Chemicals (Ann Arbor, MI, USA). HPLC grade methanol was obtained from Sigma-Aldrich (St. Louis, MO, USA). HPLC grade acetonitrile was obtained from Fisher Scientific (Pittsburgh, PA, USA). Oasis HLB was purchased from Waters Corporation (Milford, MA, USA).

Cardiac Artery Calcium Score (CACS) Evaluation

All subjects recruited for the study were offered to undergo CT evaluation. A full description of indications and potential risks of a cardiac CT were given and clearly stated in the consent form. No remuneration was offered for participating in this study. Twenty-six patients from the study population were selected on the basis of their consent to undergo the examination for the proposed studies. All these subjects were scanned with an electron beam CT scanner (Siemens Somatom Sensation 64) at the Radiology Department. No beta-blockers were administered for the scan irrespective of the individual's heart rate. Patients were placed in the supine position and positioned in the scanner so that the heart would be in the center of the scanner field. Four ECG leads were attached to each patient's chest in the standard position. The ECG was continuously recorded and stored. For calcium scoring, the CT was carried out from 1–1.5 cm below the level of the tracheal bifurcation to the diaphragm in the cranio-caudal direction and under the following conditions: tube potential 120 kV, tube variable current mAs per rotation (care dose), detector collimation 2.5 mm. A retrospective ECG-gated technique was applied to make the data reconstruction-synchronized and the CT data sets were transferred to an offline workstation with cardiac post-processing software (Syngo CT 2009 E: SYNGO Calcium Scoring).

Data analysis

Data were expressed as mean ± SD unless otherwise indicated. Group differences were tested by analysis of variance (ANOVA) or Student *t*-test as appropriate. HETEs and HOTEs differences have been checked by non-parametric test (Kruskal-Wallis Test) and differences among groups by nonparametric comparisons for each pair using Wilcoxon methods. Correlations between HDL-oxidant index and other variables were evaluated with Spearman's ρ. Statistical analysis have been performed using the JMP software (JMP 7.0)

Results

Among the D^+CVD^+ patients, 26 had had a myocardial infarction and/or had undergone coronary revascularization, 10 had peripheral artery disease, and 11 had a previous cerebrovascular event. Study groups characteristics are described in Table 1. Gender distribution was similar among groups. As compared to ND patients, the diabetic patients were older and heavier, and more frequently had hypertension. D^+CVD^+ patients, who were more often ex-smokers or current smokers, showed lower BP, total cholesterol, LDL and HDL cholesterol, but higher triglycerides. Sixty-two per cent (62%) of the diabetic patients were treated with bigunaides, 37% with sulfonylureas, 15% with glitazones, 15% with insulin, 12% with DDP4-inhibithors and 6% with incretins.

Current treatment with statins and nonsteroidal anti-inflammatory drugs increased as expected across study groups. Plasma C-reactive protein (CRP) levels were similar across groups, while erythrocyte sedimentation rate (ESR) and White Blood Cells counts (WBC) were higher in D^+CVD^+.

Lipid peroxidation products associated with HDL

The concentration of each and all lipid peroxidation products, namely, 15-HETE, 12-HETE, 5- HETE, 9-HODE, and 13-HODE, was progressively higher in HDL from ND to D^+CVD^- and D^+CVD^+ ($p<0.01$) (Table 2). Moreover, among groups, concentrations of 5-HETE, 12-HETE, 15- HETE, 13-HODE, and 9-HODE were significantly higher in HDL fractions from D^+CVD^- compared to ND patients (Fig.1). In a multivariate analysis neither smoking, nor gender nor hypertension, nor LDL cholesterol were significant predictors of any of the measured oxidized fatty acids. Diabetes was associated with higher HDL content of 5-HETE and 15-HETE when adjusted for the above mentioned confounders. The correlation between the HDL content of 15- HETE and 5-HETE is significant in the overall population ($R^2 =0.86$; $p<0.01$) as well as in the diabetic population ($R^2 =0.85$; $p<0.01$).

HDL anti-oxidant properties

HDL antioxidant properties – evaluated as the ratio between fluorescent units of test HDL to the fluorescent units obtained from sLDL (from a pool of donors) – was higher than 1 in all groups. A tendency to be higher in diabetics was observed (ND: 1.3±0.6; D^+CVD^-: 1.4±0.6; D^+CVD^+: 1.6±0.7; p=*ns*). In the whole population, there was a positive correlation between the HDL oxidant index and age ($\rho= 0.22$; $p=0.014$), and between the HDL oxidant index and HbA1c ($\rho = 0.15$; $p=0.07$). Also, a positive correlation or a positive trend between the HDL index and inflammation markers was observed: ESR$\rho = 0.19$; $p=0.03$), CRP ($\rho = 0.17$; $p=0.07$), and WBC ($\rho = 0.17$; $p=0.07$). No significant correlation was observed between the HDL index and other parameters (*i.e.*, LDL-cholesterol, triglycerides, BMI).

Coronary artery calcium score

Twenty-six patients (8 D^+CVD^-, 7 D^+CVD^+, and 11 ND) underwent cardiac CT. As expected, calcium scores were higher in patients with known CVD (CACS: ND 10±18; D^+CVD^- 457±623; D^+CVD^+; 1822±1610). Patients with pathologic coronary calcium scores (CACS>100)(19) (20) had a significantly higher HDL oxidant index and a significantly increased enrichment of 9- HODE and 13-HODE on HDL compared to patient with CACS<100 (Table 3).

Discussion

The main result of this study is that there is a progressive enrichment in oxidized fatty acids associated with HDL (isolated by ultracentrifugation in the presence of antioxidants) from patients without type 2 diabetes, with diabetes but without CVD, or diabetes with CVD (figure 1, table 2). These findings confirm previous preliminary evidence suggesting that HDL composition can be profoundly altered in patients at high cardiovascular risk and, for the first time to our knowledge, show that the change in lipid composition is more profound in patients with diabetes and clinical evidence of CVD. It is worth noting that, despite a

better cardiovascular risk profile – probably related to a more intensive pharmacologic and lifestyle intervention – D^+CVD^+ patients showed two fold more oxidized lipids associated with their HDL as compared to D^+CVD^- patients.

The presence of both HETEs and HODEs, which are enzymatically and non-enzymatically generated, on HDL indicates that lipid peroxidation occurs in response to both inflammation (cytokines) and enhanced oxidative stress from other pathways (*e.g.*, glucose and free fatty acid metabolism). It is plausible that HDL particle of different sizes may contain different amounts of oxidized fatty acids and that in type 2 diabetes HDL particles have a different size distribution. HDL particle size distribution in our subjects is presently unknown, however it should be noted that there was a tendency for all of the measured oxidized fatty acids (except 12-HETE) towards a negative association with the ratio of HDL-cholesterol to plasma apoA-I concentrations. This observation suggests that smaller particles are relatively more enriched with oxidized fatty acids. Also, there is a possibility that oxidation of lipoproteins during the lipoprotein isolation method may affect HDL lipid composition; however, it should be noted that HDL from all samples in the present study were isolated under identical conditions by ultracentrifugation. Therefore differences in oxidized fatty acids among the groups are less likely due to oxidation of lipoproteins during lipoprotein isolation.

The anti-oxidant properties of HDL studied with the DCF assay showed an increased (pro-oxidant) HDL index in all study groups. We have previously shown that the antioxidant function of poorly-controlled diabetic patients is significantly altered *viz*. healthy controls (2). In the present study, the control non-diabetic population was selected to have a similar CVD risk factor profile as the diabetic groups (*i.e.*, similar age, high blood pressure, smoking habits, lipid profile, and overweight). The partial dissociation between lipid composition and anti-oxidant function could reflect the fact that the former estimates also the exposure to oxidative stress while the latter express the ability to prevent further oxidation. In addition, the absence of a CVD event particularly in a high risk population is not necessarily associated with more extensive atherosclerosis; in fact when the study patients were grouped according to a more sensitive measurement of atherosclerosis (cardiac CT), we found that neither the HDL anti-oxidant properties nor HDL composition was altered in patients with a normal CAC score (table 3). Consistent with previous evidence in the literature (21–23) subjects in the subgroup of our population with higher risk to develop a cardiac event (i.e. CAC score greater than 100) have an atherogenic dyslipidemia. Indeed, they have higher concentration of proatherogenic lipoproteins (i.e. tg-enriched lipoprotein and lipopretein(a)) combined with lower level of commonly considered anti-atherogenic HDLs, which, according to our findings, are also dysfunctional (higher HDL index) and enriched of pro-oxidant molecules. However one of the limits of these results is that the sample size of sub-population that underwent the CT analysis does not allowed us to make strong conclusion and further studies will be needed.

In conclusion in this work we have demonstrated that patients with D^+CVD^- and D^+CVD^+ are characterized by a severe, graded enrichment of oxidized fatty acids on HDL. Taken all together our data suggests a direct relationship between CVD severity and HDL composition beyond HDL-cholesterol concentration. Thus, qualitative in addition to quantitative,

differences in these lipoproteins might be, at least in part, responsible for the “unexplained” residual risk of diabetic patients. Pharmacological normalization of HDL metabolism with concomitant correction of circulating levels, composition, and biological activities of HDL particles, with enrichment in apoA-I and reduction in HDL oxidized lipids, may constitute an efficacious therapeutic approach to attenuate atherosclerosis progression in type 2 diabetic patients. However we have limited our analysis to HETEs and HODEs in the present study and a complete lipidomics study is warranted to better understand the changes in HDL lipids in diabetic patients.

Finally a prospective study will be necessary to further confirm the relevance of HDL composition changes in the diabetic population.

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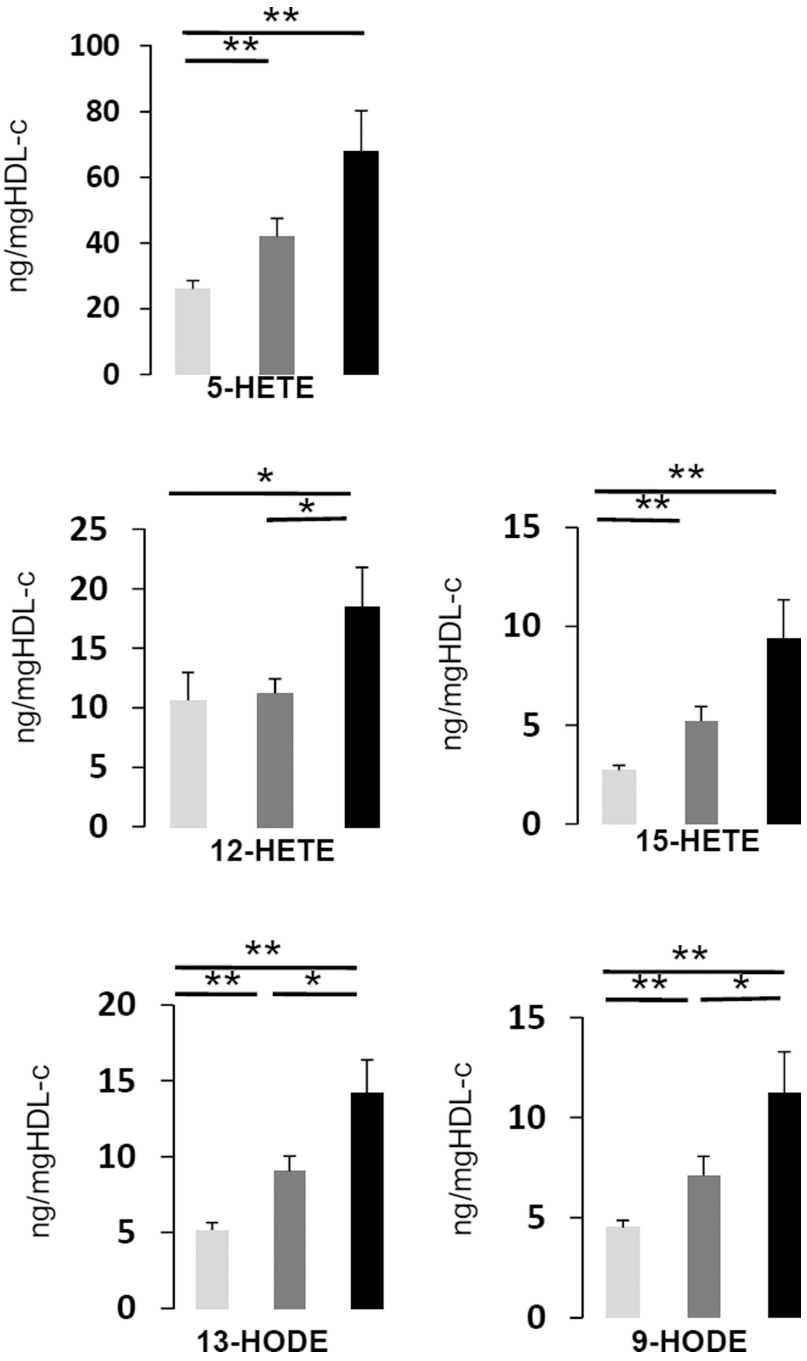

Figure 1. Oxidized lipid enrichment of HDL fractions

5-HETE,12-HETE,15-HETE,13-HODE and 9-HODE concentration evaluated by LC/MS-MS, measured on HDL isolated by ultracentrifugation from plasma of non diabetic patients (ND, n=40; white bar), diabetic patients without known cardiovascular disease (D^+CVD^-, n=40; grey bar) and diabetic patients with known cardiovascular disease (D^+CVD^+, n=38; black bar). Values are expressed as mean and SEM. *= $p<0.05$,**=$p<0.01$

Table 1

Characteristics of the study population

	No Diabetes, 40 *(ND)*	*Diabetes*, 40 *(D^+CVD^-)*	*Diabetes+CVD*,38 *(D^+CVD^+)*
Age	59±8	64±9*	66±7*
Sex (M/F)	23/17	24/16	30/8
Hypertension %	33	50	81§
SBP (mmHg)	132±27	140±16	138±18
DBP (mmHg)	86±10	84±10	81±10
Smoking (NO/EX/YES)	17/17/5	21/14/5	4/23/12*
BMI (kg/m^2)	26.3±3.7	29.1±9.2	29.5±5.0*
HbA1c (%)	5.5±0.3	7.1±1.6*	7.3±0.9*
HbA1c (mmol/mol)	37±3.3	54±17.5*	56±9.8*
Total Cholesterol (mmol/l)	5.4±0.9	5.2±0.9	4.5±0.7*
LDL –Cholesterol (mmol/l)	3.4±0.8	3.1±1.0	2.6±0.7*
HDL- Cholesterol (mmol/l)	1.5±0.3	1.4±0.4	1.2±0.2*
Triglycerides (mmol/l)	1.3±0.5	1.5±0.9	1.9±0.8*
Apolipoprotein A-I (mg/dl)	158±27	151±26	145±24
Apolipoprotein B100(mg/dl)	100±26	98±29	89±21
Lipoprotein(a) (mg/dl)	21±20	24±23	30±33
CRP (mg/dl)	0.2±0.3	0.2±0.3	0.3±0.3
ESR (mm/h)	10.1±7.1	13.7±10.4	16.2±1.6*
WBC (*10^3/μl)	6.1±1.2	6.2±1.6	7.3±2.3*§
Statins (NO/YES)	31/9	32/8	8/30§*
Fibrates (NO/YES)	0/0	0/0	0/2*§
NSAIDs (NO/YES)	38/2	36/4	10/28§*

SBP (systolic blood pressure); DBP (diastolic blood pressure); WBC (white blood cells); NSAIDs (Nonsteroidal Antiinflammatory Drugs); erythrocyte sedimentation rate (ESR)

*$p<0.05$ *vs.* ND;

§$p<0.05$ D^+CVD^+ *vs.* D^+CVD^-

Table 2

Ox-fatty acids concentration on HDL fraction from patient without diabetes (ND), with diabetes, without CVD (D^+CVD^-) and with diabetes and CVD (D^+CVD^+) expressed as median and confidence intervals (CI)).

	ND Median(CI)	D^+CVD^- Median(CI)	D^+CVD^+ Median(CI)
15-HETE	1.7(1.6–3.9)	3.5(3.6–6.8)	4.3(5.3–13.6)*
12-HETE	5.4(6.2–18.8)	8.7(88–13.9)	10.5(10.2–22.1)*
5-HETE	20.3(15.0–37.2)	33.6(30.2–53.7)	42.3(43.4–94.1)*
13-HODE	4.1(4.0–7.9)	7.4(6.9–11.2)	8.4(8.8–17.9)*
9-HODE	3.0(2.5–6.4)	5.7(5.1–9.2)	6.3(6.8–15.5)*

* $p<0.01$ (Wilcoxon/Kruskal-Wallis test)

Table 3

HDL antioxidant properties, HDL ox-lipids enrichment and blood biochemical parameters in patient with normal (CACS<100) and pathological (CACS>100) coronary artery calcium score. Values are expressed as mean±sem.

	CACS<100 (15)	CACS>100 (11)	p-value
HDL index	1.2±0.11	1.7±0.13	0.008
5-HETE (ng/mgHDL-c)	22.3±7.8	38.7±10.7	0.23
12-HETE(ng/mgHDL-c)	9.6±4.5	19.6±6.1	0.19
15-HETE (ng/mgHDL-c)	2.7±0.8	4.1±1.1	0.32
9-HODE (ng/mgHDL-c)	3.6±1.4	9.3±1.9	0.02
13-HODE (ng/mgHDL-c)	5.6±1.8	12.4±2.5	0.04
LDL-c(mmol/l)	3.3±0.15	2.8±0.18	0.06
HDL-c(mmol/l)	1.5±0.05	1.1±0.06	0.0002
Triglycerides(mmol/l)	1.3±0.1	2.1±1.2	0.0019
Lp(a) (mg/dl)	18.9±7.7	53.5±9.1	0.008