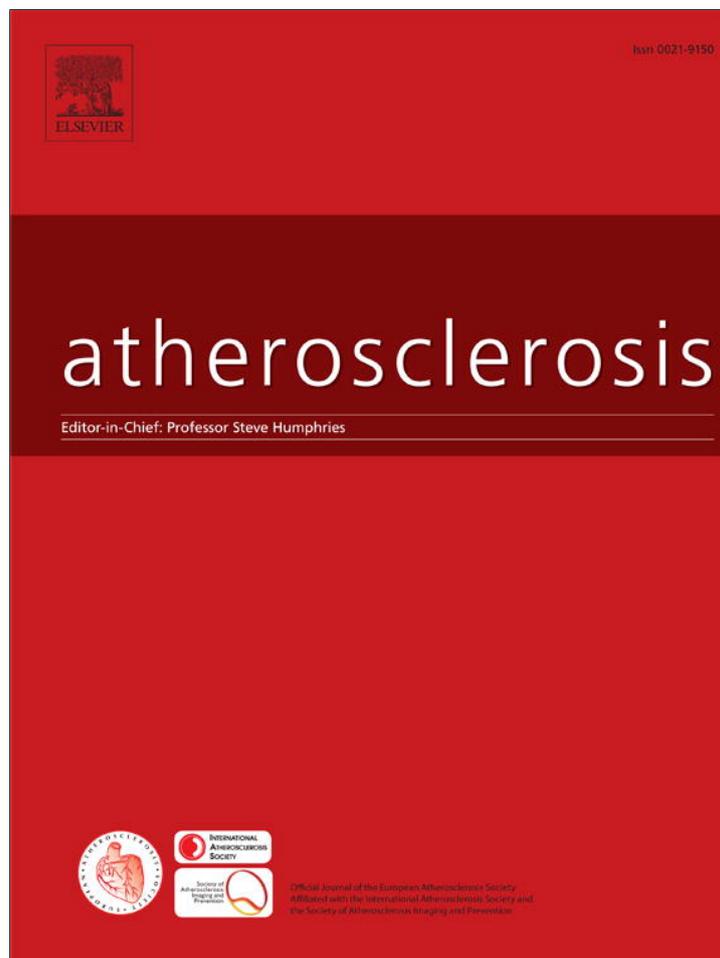


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Remarkable quantitative and qualitative differences in HDL after niacin or fenofibrate therapy in type 2 diabetic patients



Luís Masana^{a,*}, Anna Cabré^a, Mercedes Heras^a, Núria Amigó^b, Xavier Correig^b, Sergio Martínez-Hervás^c, José T. Real^c, Juan F. Ascaso^c, Helena Quesada^e, Josep Julve^{d,e}, Xavier Palomer^f, Manuel Vázquez-Carrera^f, Josefa Girona^a, Núria Plana^a, Francisco Blanco-Vaca^{d,e}

^a Vascular Medicine and Metabolism Unit, Research Unit on Lipids and Atherosclerosis, "Sant Joan" University Hospital, Universitat Rovira i Virgili, IISPV, Spanish Biomedical Research Centre in Diabetes and Associated Metabolic Disorders (CIBERDEM), Reus, Spain

^b Metabolomics Platform and Center for Omic Sciences, Universitat Rovira i Virgili, IISPV, CIBERDEM, Reus, Spain

^c Endocrinology and Nutrition Department, Hospital Clínico Universitario, CIBERDEM, INCLIVA, Department of Medicine, University of Valencia, Valencia, Spain

^d Department of Biochemistry and Molecular Biology, Universitat Autònoma de Barcelona, Barcelona, Spain

^e Institut d'Investigació Biomèdica Sant Pau (IIB Sant Pau), CIBERDEM, Barcelona, Spain

^f Department of Pharmacology and Therapeutic Chemistry, Institut de Biomedicina de la Universitat de Barcelona (IBUB) and CIBERDEM, Faculty of Pharmacy, University of Barcelona, Barcelona, Spain

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ABSTRACT

HDL-increasing drugs such as fenofibrate and niacin have failed to decrease the cardiovascular risk in patients with type 2 diabetes. Drug-mediated quantitative and qualitative HDL modifications could be involved in these negative results. To evaluate the quantitative and qualitative effects of niacin and fenofibrate on HDL in patients with type 2 diabetes, a prospective, randomised controlled intervention trial was conducted. Thirty type 2 diabetic patients with low HDL were randomised to receive either fenofibrate (FFB) or niacin + laropirant (ERN/LRP) as an add-on to simvastatin treatment for 12 weeks according to a crossover design. At the basal point and after each intervention period, physical examinations and comprehensive standard biochemical determinations and HDL metabolomics were performed. Thirty nondiabetic patients with normal HDL were used as a basal control group. ERN/LRP, but not FFB, significantly increased HDL cholesterol. Neither ERN/LRP nor FFB reversed the HDL particle size or particle number to normal. ERN/LRP increased apoA-I but not apoA-II, whereas FFB produced the opposite effect. FFB significantly increased Preβ1-HDL, whereas ERN/LRP tended to lower Preβ1-HDL. CETP and LCAT activities were significantly decreased only by ERN/LRP. PAF-AH activity in HDL and plasma decreased with the use of both agents. Despite their different actions on antioxidant parameters, none of the treatments induced detectable antioxidant improvements.

ERN/LRP and FFB had strikingly different effects on HDL quantity and quality, as well as on HDL cholesterol concentrations. When prescribing HDL cholesterol increasing drugs, this differential action should be considered.

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1. Introduction

Cardiovascular (CV) diseases are responsible for approximately

50% of deaths in patients with type 2 diabetes [1]. In the presence of additional CV risk factors, achieving a low density lipoprotein (LDL) concentration below 70 mg/dl is recommended [2]. Even if the LDL target is achieved, an important residual risk remains. A portion of this residual risk has been attributed to lipid profile alterations, as well as plasma LDL concentrations [3]. Patients with type 2 diabetes usually have profound lipid metabolism derangement, which is characterised by low high density lipoprotein (HDL) and high

* Corresponding author. Vascular Medicine and Metabolism Unit, Research Unit on Lipids and Atherosclerosis, Sant Joan University Hospital, Universitat Rovira i Virgili, C. Sant Joan S/N, 43201 Reus, Spain.

E-mail address: luis.masana@urv.cat (L. Masana).

triglyceride concentrations. This lipid pattern is referred to as atherogenic dyslipidemia because of its high vascular damaging capacity. The inverse association between circulating HDL cholesterol concentrations and CV disease risk is unquestionable [4]. In many epidemiological studies, HDL cholesterol below 40 mg/dl in men and 45 mg/dl in women has been associated with an increased CV disease risk [2]. Recent data from the “Emerging Risk Factors Collaboration” confirmed that HDL cholesterol is inversely associated to coronary heart disease after adjusting for lipid and non-lipid risk factors [5].

Despite this strong epidemiological association, increasing HDL cholesterol by medications has not produced a beneficial impact on CV disease risk. In recent years, clinical outcome intervention trials using fibrates, niacin and cholesteryl ester transfer protein (CETP) inhibitors have had negative results [6]. Among the fibrate intervention trials, only the VA-HIT study using gemfibrozil showed a 22% relative CV disease risk reduction associated with a 6% increase in HDL cholesterol [7]. More recent studies using fenofibrate (FIELD and ACCORD) [6,8] did not show a beneficial effect, albeit post hoc analyses suggested a marginal benefit in the atherogenic dyslipidemia subgroup. Similarly, two studies that used niacin as an add-on to statin treatment, AIM-HIGH and HPS2-THRIVE, were prematurely stopped due to a lack of efficacy [9,10]. In all of these trials, the effect on HDL cholesterol concentrations was relatively poor, with mean increases ranging from 0% to 6%. The failure of the fibrate and niacin trials has been attributed to a lack of effect on lipid parameters or a poor study design, among other reasons. Beyond these circumstances, the complex composition and metabolism of HDL particles must be considered. Data from proteomic and lipidomic studies have shown the heterogeneity of this lipoprotein family, which is involved in many biological functions [11–15]. Moreover, HDL has considerable plasticity and is capable of changing its composition according to the environmental needs. Although reverse cholesterol transport is considered to be the key HDL antiatherogenic function, other biological effects of HDL are equally important, including its anti-inflammatory, endothelial protective, and antioxidant capacities [16]. The cholesterol content in HDL is only a subrogated marker of the HDL particle concentration and has a weak correlation with HDL functions [17]. The anti-inflammatory, antioxidant, and endothelial protective or antiapoptotic effects of HDL seem to be more related to the HDL particle shape, size, number and composition. All of these characteristics are altered by pathological conditions such as type 2 diabetes mellitus (T2DM) [18]. Many efforts have been made to evaluate the clinical impact of HDL function rather than HDL cholesterol concentrations. Recently, the HDL cholesterol efflux capacity was observed to be a better indicator of HDL CV protection than HDL cholesterol [19]. Despite this evidence, HDL cholesterol concentrations remain the primary treatment determinant, and the efficacy of medication is assessed by its capacity to increase HDL cholesterol.

In this study, we hypothesized that global HDL particle alterations of T2DM patients are reversed by neither ERN/LRP nor FFB despite their HDL cholesterol increasing effect and that both drugs impact differently in HDL particle size distribution, composition and HDL metabolic determinants in these patients.

2. Patients and methods

2.1. Subjects and design of the study

Thirty type 2 diabetic patients, 19 male and 11 female, ranging in age from 30 to 70 years old and with HDL not exceeding 50 mg/dl in men or 60 mg/dl in women were recruited. This HDL cut off points were selected to avoid the impact of genetic factors associated to

high HDL values. The exclusion criteria were as follows: smoker, diagnosed with diabetes less than three months before, triglyceride levels above 400 mg/dl, glycated haemoglobin higher than 9%, albuminuria above 300 mg/mg creatinine, chronic kidney disease (estimated glomerular filtration rate <30 ml/min/1.73 m²), advanced retinopathy, neuropathy, cardiovascular disease in the last three months, chronic liver insufficiency, neoplastic disease or any chronic or incapacitating disease. The control group consisted of 30 age- and gender-matched subjects without diabetes and with HDL cholesterol higher than 40 mg/dl for men or 50 mg/dl for women. After a 6-week lipid-lowering drug wash-out, the patients with type 2 diabetes were randomly distributed into two groups. One group received 20 mg simvastatin plus 145 mg fenofibrate, and the other group received 20 mg simvastatin plus 2 g niacin plus laropiprant for a 12-week period. After this intervention period, the patients followed a new 6-week lipid-lowering drug wash-out; subsequently, they were shifted to the other lipid-lowering drug, in a crossover design, for a 12-week period (Fig. 1). Physical examinations, anthropometry and blood extraction for standard biochemical and metabolic tests were obtained at the basal point and after each intervention period in the type 2 diabetes group and at the basal point in the control group. All of the study investigations were conducted according to the principles expressed in the Declaration of Helsinki. The study was approved by the Ethic Committees of the recruiting hospitals. All of the subjects provided their written informed consent before participating in the study.

2.2. Sample collection and storage

At the time points indicated in the flow chart (Fig. 1), fasting blood samples were collected in serum tubes with EDTA and were centrifuged immediately at 1500 g for 15 min at 4 °C. Aliquots of plasma and serum were stored at –80 °C until the analyses were performed (except for pre β 1-HDL).

2.3. Standard lipid analyses

Biochemical parameters, lipids, apolipoproteins, fructosamine and homocysteine were measured using colourimetric, enzymatic and immunoturbidimetric assays (Spinreact, SA, Spain; Wako Chemicals GmbH, Germany; Polymedco, NY) adapted to a Cobas Mira Plus autoanalyser (Roche Diagnostics, Spain) [20–22].

Enzymes and protein concentrations are outlined in the [supplemental materials](#).

2.4. Pre β 1-HDL measurements

To determine pre β 1-HDL measurements, plasma samples were immediately placed on ice in a 50% sucrose solution. Pre β 1-HDL was analysed using a quantitative ELISA (Daichii, Japan).

2.5. HDL isolation using ultracentrifugation

Total HDL was isolated from plasma using sequential preparative ultracentrifugation (uc) at 1.21 mg/dl density according to previously described techniques [23]. Ultracentrifuged HDL (ucHDL) fractions were stored at –80 °C until biochemical studies were performed.

2.6. Plasma and HDL oxidation status

The OxyStat (Biomedica, Wien) colorimetric assay was used for the quantitative determination of lipid peroxides in apoB-depleted plasma after the precipitation of β -lipoproteins using phosphotungstic acid and magnesium ions (Roche Diagnostics). The results

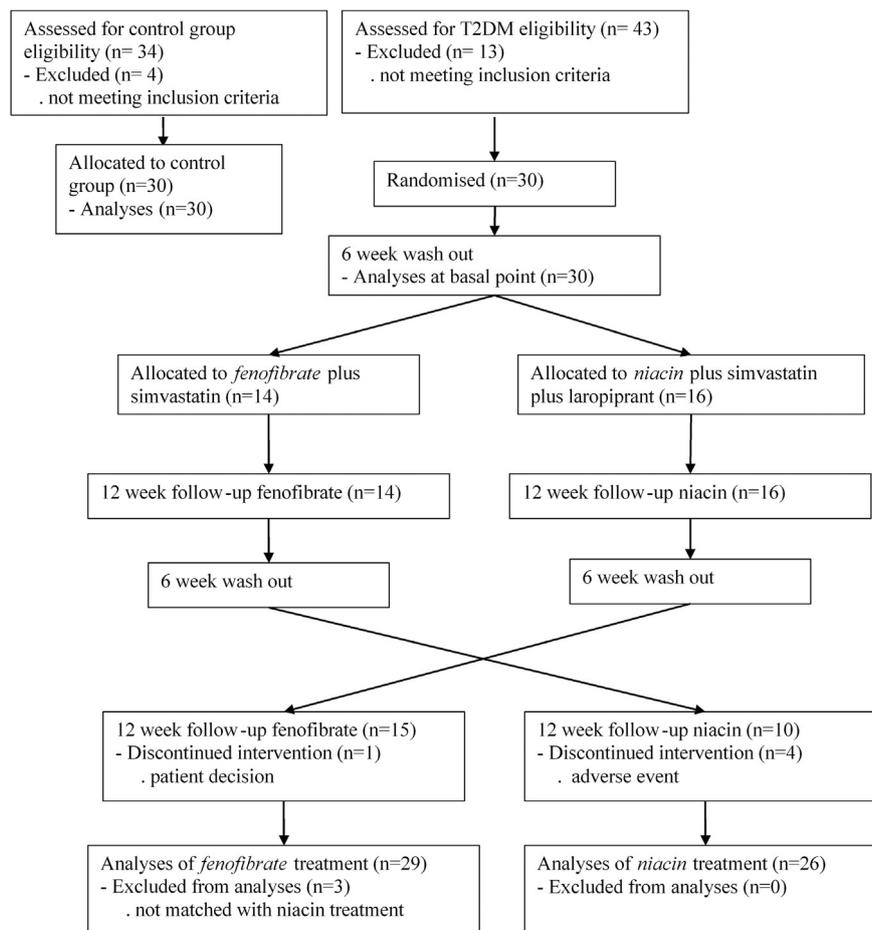


Fig. 1. Flow chart of participant enrolment, randomisation and analysis.

are expressed as μmol lipoperoxides/mg apoA-I.

Serum paraoxonase 1 (PON1) and paraoxonase 3 (PON3) concentrations were determined using an in-house ELISA and rabbit polyclonal antibodies generated against synthetic peptides with sequences specific for mature PONs. The employed peptides were CRNHQSSYQTRLNALREVQ (specific for PON1) and CRVNASQVEPVEPEN (specific for PON3). The details of these methods have been previously reported [24]. Serum PON1 lactonase activity was analysed by measuring 5-thiobutyl butyrolactone (TBBL) hydrolysis, as previously described [25,26].

HDL antioxidant activity was determined by conjugated diene formation by incubating the patient's HDL (0.1 mg/ml apoA1) with human LDL (0.1 mg/ml apoB, obtained from a pool of normolipidemic individuals) in the presence of 2.5 $\mu\text{mol/L}$ CuSO_4 . Continuous monitoring at an absorbance of 234 nm was performed in a microplate reader (BioTek Synergy, Winooski, VT, USA) at 37 °C for 4 h. The kinetics of LDL in the LDL + HDL incubations were calculated by subtracting the kinetics of HDL incubated without LDL; the lag phase was calculated as previously described [27].

2.7. HDL composition analyses

In the uHDL fraction, cholesterol, triglyceride, total protein, phospholipid, apolipoprotein AI (apoA-I) (Roche Diagnostics), apolipoprotein A-II (apoA-II), apolipoprotein E (apoE), apolipoprotein CII (apoC-II), and apolipoprotein CIII (apoC-III) (Kamiya Biomedical Company) contents were quantified using enzymatic and nephelometric assays adapted to a BM/HITACHI 911

autoanalyser (Spinreact S.A.U., Spain).

2.8. HDL analyses by 2D diffusion-ordered ^1H NMR spectroscopy (DOSY)

The uHDL fraction samples were analysed using nuclear magnetic resonance (NMR) spectroscopy and a modified existing protocol [28]. The ^1H NMR spectra were recorded using a BrukerAvance III spectrometer at 310 K. We used the double stimulated echo (DSTE) pulse program with bipolar gradient pulses and a longitudinal eddy current delay (LED). The DSTE methyl signal was fitted with one lorentzian function to obtain the averaged diffusion coefficient of the lipoprotein particles. The hydrodynamic radii of the lipoprotein fractions were extracted from the Stokes-Einstein equation. Further details about the uHDL NMR feature extraction and HDL particle size distribution and number calculations are outlined in the [supplemental material](#).

2.9. Statistical analysis

Normal distributed data are shown as the mean \pm SD values, and non-normal distributed data are shown as the median (interquartile range). We performed two different statistical tests to detect differences between the studied variables. A statistical Mann–Whitney U test was performed to identify significant differences between the control group and the group comprising patients with type 2 diabetes, followed by a Wilcoxon signed-rank test to evaluate the treatment effects for paired samples. We

performed alpha corrections due to multiple testing by multiplying the *p* value by the number of related variables tested (lipids, enzymes, oxidation, HDL subclasses). We excluded any carryover effect by the Fleiss method. There were no significant differences (by *t* test) in the results obtained in any of the variables after the same treatment, regardless of the intervention order [29]. Subsequently, the data of the two sequences were combined and analysed as described in the design section. The analyses were performed using SPSS software (IBM SPSS Statistics, version 20). *P* < 0.05 was considered to be statistically significant.

3. Results

3.1. Baseline differences

The anthropometric and clinical characteristics of these groups are presented in Supplemental Table 1S. In Table 1, we show the lipid metabolism and oxidation parameters and HDL subclass distribution in both T2DM patients and controls. As expected, the patients with T2DM had lower HDL cholesterol and ApoA1. The total number of HDL particles was lower in T2DM patients (*P* = 0.009). The difference was primarily due to the medium-sized HDL particles (*P* = 0.004) (Table 1). They had higher preβ1-HDL and CETP activity and lower PON1. HDL from T2DM patients had less cholesterol and apo E and more triglycerides and apoC-III (Supplemental Table 2S). Because of the side effects of the medications, one patient withdrew from the study during the fenofibrate treatment, and four patients withdrew during the niacin treatment. The anthropometric and clinical characteristics of these groups are presented in Supplemental Table 3S.

Table 1
Biochemical characteristics of the control population and T2DM patients.

	Control (n = 30)	T2DM (n = 30)	<i>P</i>
Lipoproteins			
Cholesterol (mmol/L)	5.42 ± 0.99	6.14 ± 1.24	0.030^a
Triglycerides (mmol/L)	0.95 (0.73–1.20)	2.39 (1.63–3.49)	<0.001
HDL-C (mmol/L)	1.51 ± 0.36	1.05 ± 0.28	<0.001
ApoA-I (g/L)	149 ± 15	132 ± 15	<0.001
ApoA-II (g/L)	30.3 ± 3.9	28.6 ± 4.8	0.095
ApoB-100 (g/L)	96 ± 22	121 ± 26	0.001
preβ1-HDL (μg/ml)	17.8 (11.6–22.6)	22.8 (15.5–27.7)	0.027^a
Enzymes			
CETP mass (μg/ml)	2.5 ± 0.7	2.5 ± 0.7	0.906
CETP activity (pmol/h*μl)	7.2 ± 2.0	9.2 ± 2.9	0.006
LCAT mass (μg/ml)	9.6 ± 1.4	10.7 ± 1.9	0.006
LCAT activity (FER)	6.28 ± 4.6	11.3 ± 10.6	0.152
PAF-AH (μmol/min*ml)	19.7 ± 5.1	22.7 ± 7.5	0.101
PAF-AH in HDL (μmol/min*ml)	11.9 ± 4.9	12.6 ± 5.8	0.644
Oxidation			
PON1 (mg/L)	78.6 ± 23.9	62.0 ± 23.4	0.007
PON3 (mg/L)	1.4 ± 0.5	1.6 ± 0.5	0.255
Lactonase activity (U/L)	6.4 ± 2.9	7.6 ± 2.1	0.021^a
Paraoxonase activity (U/L)	269.2 ± 101.9	320.2 ± 146.4	0.193
LOOH in HDL (μmol/mg)	0.15 ± 0.10	0.19 ± 0.10	0.044^a
Antioxidant capacity (% dienes)	100.1 ± 21.7	91.6 ± 25.9	0.208
HDL subclass particle concentration (μmol/L)			
Large HDL	0.6 (0.4–0.8)	0.5 (0.4–0.7)	0.196
Medium HDL	3.9 (2.9–5.3)	2.9 (1.8–4.2)	0.004
Small HDL	7.8 (6.1–9.7)	7.8 (6.2–10.2)	1
Total HDL	13.6 (11.0–15.2)	10.8 (10.0–12.6)	0.009

Normal distributed data are shown as the mean ± SD values and non-normal distributed data as the median (interquartile range). The control population and T2DM patients were analysed using the Mann–Whitney *U* test.

Bold signifies *P* value.

^a Results lost statistical significance at *P* ≤ 0.05 after we adjusted for α inflation caused by multiple testing.

3.2. Changes induced by fenofibrate

Despite a significant decrease in plasma TG, FFB did not increase HDL cholesterol or apoA-I (Table 2). Conversely, it increased apoA-II and preβ1-HDL (Table 2) and decreased HDL apoC-III whereas HDL apoE remained unchanged (Supplemental Table 4S). LCAT and CETP activities did not vary with treatment. FFB decreased the number of medium size HDL particles (Table 2 and Fig. 2).

Regarding oxidative parameters, FFB decreased both paraoxonase and PAF-AH activities without significant changes in LOOH and antioxidant capacity (Table 2).

3.3. Changes induced by niacin

ERN/LRP significantly increased HDL cholesterol and apoA-I, and showed a tendency to decrease preβ1-HDL (Table 2). CETP and LCAT mass and activity were decreased after treatment. ERN/LRP did not show any effect on HDL subclass distribution (Table 2 and Fig. 2).

Regarding oxidation, PAF-AH and paraoxonase activities were significantly decreased by ERN/LRP (Table 2).

3.4. Comparison between treatments

Although ERN/LRP significantly increased HDL cholesterol, the total HDL particle number was not significantly modified by any of the treatments (Table 2).

The HDL spectra of medium and large HDL particles were different between controls and T2DM and were not reversed to normal after any treatment (Fig. 3A). No differences in the NMR spectrum of small HDL were observed. Fig. 3B shows the average HDL particle sizes, confirming that the HDL from T2DM patients is smaller than that of controls, and neither FFB nor ERN/LRP fully correct this alteration. The mean radius for the healthy group (4.7 nm) was higher than that of the T2DM group (4.5 nm) (*P* = 0.002). FFB tended to shift the distribution towards smaller particles, whereas RN/LRP treatment increased the relative concentration of the medium HDL subclass, which consequently, although not significantly, approached the healthy state. The effects of different treatments on the mean HDL radius were significantly different (*P* = 0.042).

4. Discussion

Three essential messages are obtained from our study. The first message is that pharmacological intervention with ERN/LRP and FFB in T2DM patients leads to important HDL particle modifications, beyond HDL cholesterol concentrations. The second message is that these composition changes differ according to the HDL cholesterol-increasing medication used. The third message is that neither ERN/LRP nor FFB reverses diabetic HDL alterations *ad integrum*. These differences could, in part, be explained by the mechanisms of action of both medications. FFB is a PPAR α agonist that increases proteins associated with lipolysis activity, whereas niacin, among other mechanisms, reduces adipose tissue lipolysis [30,31]. The post-treatment HDL particles differ depending on the medication used, and they also differ from normal; therefore, the expected effect on cardiovascular risk should be unequal. HDL cholesterol, which is the primary variable that is expected to be modified by ERN/LRP and FFB, increases in different proportions. Although niacin produced a mean 18% increase, FFB did not significantly modify HDL cholesterol. These results are in concordance with previous publications that show similar effects of niacin and FFB [6,8–10]. Niacin was also the only medication that produced a significant increase in apoA-I. However, consistent with previous observations, only FFB induced a significant 15% increase

Table 2
Biochemical characteristics at baseline and post ERN/LPR and FFB treatments in T2DM patients.

	T2DM baseline (n = 26)	T2DM Post-FFB (n = 26)	P FFB vs. baseline*	T2DM post-ERN/LPR (n = 26)	P ERN/LPR vs. baseline*	P between treatments**
Lipoproteins						
Cholesterol (mmol/L)	6.17 ± 1.27	4.50 ± 0.96	<0.001	4.41 ± 0.77	<0.001	0.818
Triglycerides (mmol/L)	2.50 (1.98–4.20)	1.86 (1.38–2.62)	0.006	1.50 (1.07–2.35)	0.003	0.058
HDL-C (mmol/L)	1.03 ± 0.29	1.03 ± 0.28	0.620	1.22 ± 0.36	0.003	0.022 ^a
ApoA-I (g/L)	1.32 ± 0.16	1.33 ± 0.16	0.284	1.37 ± 0.19	0.032	0.022 ^a
ApoA-II (g/L)	0.285 ± 0.050	0.327 ± 0.054	<0.001	0.285 ± 0.047	0.576	<0.001
ApoB-100 (g/L)	1.20 ± 0.27	0.93 ± 0.24	<0.001	0.85 ± 0.22	<0.001	0.011 ^a
preβ1-HDL (μg/ml)	25.5 (16.5–27.9)	28.8 (22.4–37.8)	0.005	21.3 (17.7–29.1)	0.603	0.016 ^a
Enzymes						
CETP mass (μg/ml)	2.6 ± 0.7	2.1 ± 0.5	<0.001	2.1 ± 0.6	<0.001	0.435
CETP activity (pmol/h*μl)	9.6 ± 2.9	8.3 ± 2.0	0.080	7.8 ± 2.0	0.004	0.065
LCAT mass (μg/ml)	10.6 ± 2.0	9.7 ± 1.8	<0.001	9.9 ± 2.3	0.025 ^a	0.559
LCAT activity (FER)	11.8 ± 10.8	7.7 ± 8.1	0.180	3.8 ± 5.6	0.010 ^a	0.046 ^a
PAF-AH (μmol/min*ml)	22.2 ± 7.6	18.1 ± 6.9	0.006	17.8 ± 6.7	0.020 ^a	0.663
PAF-AH in HDL (μmol/min*ml)	12.2 ± 5.7	10.0 ± 3.5	0.008	9.6 ± 4.2	0.007	0.463
Oxidation						
PON1 (mg/L)	63.2 ± 24.7	73.7 ± 27.2	0.015 ^a	65.5 ± 23.6	0.248	0.060
PON3 (mg/L)	1.6 ± 0.5	1.5 ± 0.7	0.865	1.4 ± 0.5	0.135	0.196
Lactonase activity (U/L)	7.2 ± 1.6	6.2 ± 2.3	0.065	6.8 ± 2.4	0.469	0.339
Paraoxonase activity (U/L)	292.4 ± 114.1	265.1 ± 92.1	0.023 ^a	273.9 ± 105.9	0.020 ^a	0.716
LOOH in HDL (μmol/mg)	0.20 ± 0.11	0.19 ± 0.10	0.638	0.23 ± 0.12	0.086	0.046 ^a
Antioxidant capacity (% dienes)	95.0 ± 22.9	87.4 ± 44.6	0.226	96.1 ± 38.4	0.778	0.128
HDL subclass particle concentration (μmol/L)						
Large HDL	0.5 (0.4–0.7)	0.5 (0.2–0.7)	0.909	0.6 (0.4–0.8)	0.114	0.029 ^a
Medium HDL	3.3 (2.1–4.3)	2.4 (1.9–3.2)	0.044 ^a	3.3 (2.4–3.9)	0.361	0.032 ^a
Small HDL	7.8 (6.0–10.0)	8.1 (6.4–9.1)	0.989	7.1 (5.7–8.4)	0.301	0.139
Total HDL	10.8 (9.8–12.5)	10.6 (9.6–13.2)	0.395	10.9 (8.9–13.0)	0.346	0.986

Normal distributed data are shown as the mean ± SD values and non-normal distributed data as the median (interquartile rang). Pre-treatment baseline data were compared with post-treatment data using the paired sample Wilcoxon signed rank test; *P, the effect of treatment was analysed using the paired sample Wilcoxon signed rank test; **P. Bold signifies P value.

^a Results lost statistical significance at P ≤ 0.05 after we adjusted for α inflation caused by multiple test.

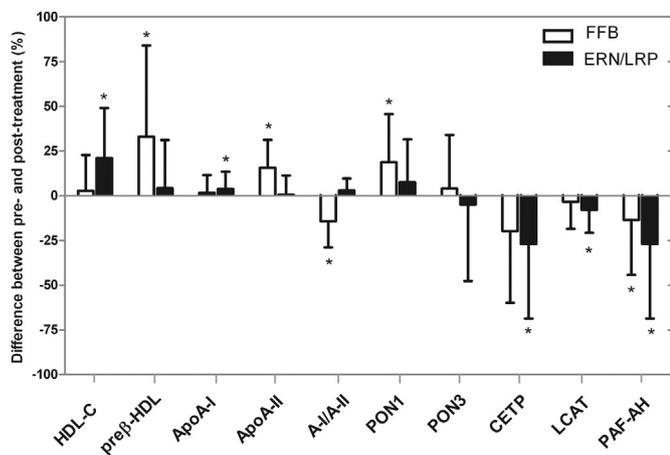


Fig. 2. Percentage of change between baseline and post-FFB and post-ERN/LRP of HDL lipids, apolipoproteins and enzymes. The data represent the mean ± SD values; n = 26. The effect of treatment was analysed using the paired sample Wilcoxon signed rank test, *P < 0.05.

in apoA-II [32]. Patients with T2DM had significantly higher preβ1-HDL particles, which were further increased by FFB, whereas niacin tended to decrease the levels of preβ1-HDL. The clinical repercussion of this fact is not clear. Both high and low levels of preβ1-HDL have been associated with cardiovascular risk and the presence of cardiovascular risk factors [33,34]. These discrepancies can be explained by different mechanisms, including increased synthesis, decreased maturation, or both, that can be involved in the origin of preβ1-HDL plasma accumulation. Patients with T2DM had higher

CETP activity, which tended to be reduced by both treatments, although only after ERN/LRP, this tendency was statistically significant, which suggests that partial inhibition of CETP could be a mechanism that explains the effects of both ERN/LRP and FFB on HDL cholesterol concentrations. T2DM patients had a high LCAT mass, but no significant differences in activity were observed. ERN/LRP significantly reduced LCAT activity in T2DM patients. Despite a different marginal effect on PAF-AH and PON 1 mass and activity, neither fenofibrate nor niacin treatment was associated with better oxidation profile markers according to the lipoperoxide concentration in apoB-lipoprotein-containing depleted plasma, which is an indirect index of HDL oxidation, and the capability of HDL to protect against LDL oxidation. Diabetic patients had an HDL fraction with increased proportions of apoA-II and C-III and half the concentration of apoE. Although FFB increased apoA-II, niacin reduced its concentration. FFB reduced apoC-III; however, neither FFB nor ERN/LRP modified the proportion of apoE. The biological impact of these differences is not known (Supplemental Tables 3S and 4S). We speculate that the increased apoA-II after FFB treatment may modify apolipoprotein exchange between HDL and triglyceride-rich particles, thus decreasing lipoprotein lipase activity and influencing both triglyceride and HDL cholesterol concentrations [35].

The NMR results reinforced that HDL from T2DM patients is clearly different from the healthy group in terms of particle size and number. T2DM patients had fewer HDL particles, and the HDL particles from T2DM patients had smaller radii. These alterations were not reversed by ERN/LRP or FFB.

Some limitations of our study are that the intervention period of the study was only 12 weeks; therefore, our results cannot be extrapolated over a longer period of time. The sample size is small

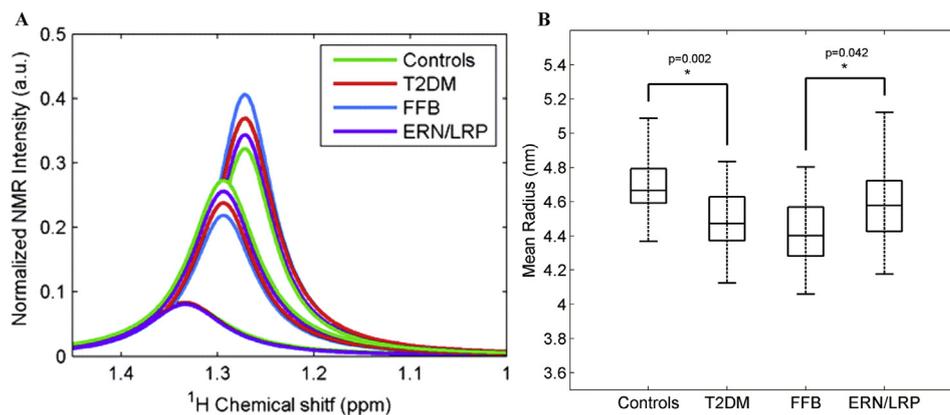


Fig. 3. HDL particle size distribution in controls and T2DM patients before and after ERN/LRP and FFB treatments. A) The coloured curves are the means of the three normalised functions for each group. The area under each curve represents the relative lipid concentration of a particular subclass. B) The mean HDL radius in controls and T2DM patients before and after ERN/LRP and FFB treatments. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

due to the comprehensive analyses performed, including metabolomics techniques. This rather small sample size allows only the detection of large effects; however, it does warrant enough power for the main results of the study. The ERN preparation was associated with LRP, so we cannot exclude the lipid effects associated with this product, although if they exist, they seem to be very light.

ERL/LRP has been withdrawn from the market, although other niacin-based pills are available in different countries, and FFB is widely available. The overall conclusions of our work are that neither ERN/LPP nor FFB reverse HDL particle abnormalities associated to T2DM. Moreover these two drugs act differently on HDL. Our results should contribute to a better understanding of the negative results observed in randomized controlled trials using niacin or fenofibrate. In our hands these two drugs don't improve HDL particle composition, size and metabolism, despite a marginal impact on HDL cholesterol concentrations. Clinicians prescribing these drugs must be aware of their overall impact on HDL particles and lipoprotein metabolism.

Conflict of interest

None declared.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at <http://dx.doi.org/10.1016/j.atherosclerosis.2014.12.006>.

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