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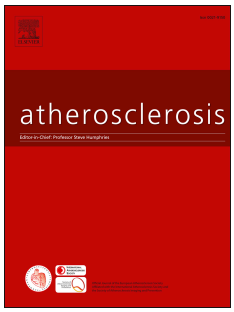

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Cholesterol efflux capacity of large, small and total HDL particles is unaltered by atorvastatin in patients with type 2 diabetes

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Abstract

Background and aims: Research on the biologic activities of HDL, such as cholesterol efflux capacity and HDL composition, have allowed the understanding of the effect of interventions directed to improve cardiovascular risk. Previously, statin therapy has shown conflicting results in its effects on cholesterol efflux capacity of HDL, the underlying mechanisms are unclear but studies with positive effect are associated with an increase of HDL-cholesterol levels. We investigated if 10 weeks with atorvastatin therapy changes HDL efflux capacity and the chemical composition of its subpopulations.

Methods: In a before-after design basis, HDL-cholesterol levels, chemical composition and cholesterol efflux capacity from HDL subpopulations isolated by isophynic ultracentrifugation were assessed in plasma samples from 60 patients with type 2 diabetes mellito (T2DM) at baseline and after 10 weeks of treatment with 20 mg of atorvastatin. Cholesterol efflux was measured from human THP-1 cells using large, light HDL2b and small, dense 3c subpopulations as well as total HDL as acceptors. Changes of cholesterol efflux and chemical composition of HDL after treatment were analyzed. Correlations among variables potentially involved in cholesterol efflux were evaluated.

Results: A significant decrease of 4% in HDL-cholesterol levels was observed from 47 (42-54) to 45 (39-56) mg/dL, p= 0.02. Cholesterol efflux from total-HDL and HDL2b and 3c subfractions was maintained unchanged after treatment. The total mass of HDL remained unaffected, except for HDL3a subpopulation accounted for by a significant increase in total protein content. No significant correlations for variables previously known to be associated with cholesterol efflux were found in our study.

Conclusions: Short therapy of 10 weeks with 20 mg of atorvastatin does not modify the cholesterol efflux capacity neither the total mass of HDL2b, HDL3c and total HDL. The

discrepancy with previous reports may be due to selective effects among different classes of statins or differences in the approaches to measure cellular cholesterol efflux.

Key words:_Cholesterol efflux, HDL subpopulations, Type 2 diabetes, Atorvastatin, HDL chemical composition

Introduction

Anti-atherogenic activities and chemical composition of high-density lipoprotein (HDL) are altered in patients with Type 2 diabetes (T2DM)[1][2][3][4][5][6][7][8][9][10]. Diabetic dyslipidemia is characterized by high levels of triglyceride-rich lipoproteins, low concentrations of HDL-cholesterol (HDL-C) and elevated proportions of small, dense LDL particles (sdLDL). Although the pathophysiology of diabetic dyslipidemia is not clearly elucidated yet, insulin-resistant state and elevated activities of key proteins regulating HDL and LDL metabolism, and notably cholesteryl ester transfer protein (CETP) and hepatic lipase, play an important role[11][12][13].

Among their anti-atherogenic effects, HDL particles are able to stimulate cholesterol efflux from peripheral cells, the first step of the reverse cholesterol transport pathway. HDL capacity to act as an acceptor for free cholesterol depends on the integrity of specific cellular cholesterol export pathways which involve ATP-binding cassette transporter (ABC) A1, ABCG1, scavenger receptor class B type I (SR-BI) and passive diffusion[14][15][16][17][18][19]. Currently, several assays have been described to determine the cholesterol efflux capacity, most of them using cellular lines from murine and human macrophages, tritiated cholesterol and Apo-B depleted-serum, HDL or ApoA-1 as cholesterol acceptors[20][21]. The recognition of different HDL subpopulations has allowed the identification of particles with diverse degrees of efficacy, potentially accounted by a particular distribution of their chemical component and biologic activity [22][23][24][25][26]. Interestingly, liquid chromatography-mass spectrometry (LC-MS) analysis has documented profound alterations in the HDL lipidome in patients with T2DM, suggesting that the structure of HDL is under continuing remodeling in this disease and that

HDL lipidomics can equally contribute to identify biomarkers of normal and deficient HDL functionality[7][27][28]. In the last years, focus has been directed to therapies to improve HDL-C and more recently HDL function. Therapies that increase HDL-C has failed to show a diminution in cardiovascular risk and interventional studies directed to demonstrate an improvement in HDL functions have produced conflicting results[8][29][30][31][32][33][34] [35][36][37]. Recent studies revealed that cholesterol efflux capacity was inversely associated with the incidence of cardiovascular events in a population-base cohort study [38]. This report highlights the relevance of studying HDL function in high-risk populations.

Current guidelines recommend early use of lipid-lowering drugs, particularly statins, in a large proportion of patients with T2DM as a key approach to reduce cardiovascular morbidity[39][40][41]. Despite that, cardiovascular mortality remains the leading cause of death in T2DM, even in cases treated in accordance with current recommendations. Statins are 3-hydroxi-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitors. Early blocking of this microsomal enzyme reduces hepatic cholesterol synthesis and promotes a faster clearance of circulating cholesterol. In numerous large-scale studies statins have proven to reduce the risk of acute cardiac events and death[42][43]. Previous reports, which included T2DM patients as a target population, brought about contradictory results in terms of the capacity of statins to improve anti-atherogenic activities of HDL, such a cholesterol efflux, anti-inflammatory activity and anti-oxidative capacity[44][45][46][18] Moreover, recent kinetic studies have reported that most of the effects of statins are molecule-specific rather class-related and that statins have differential effects in HDL cholesterol concentration and HDL functions[47][48]. Most of the studies of the effect of statins on

HDL function have been conducted in patients with preexisting cardiovascular disease (CAD). Except for one small study on HDL cholesterol efflux after treatment with simvastatin, T2DM patients have not been a matter of a properly controlled study.

We conducted a single group, before-after study to evaluate the effect on HDL function and composition of a 10-week treatment with atorvastatin of T2DM patients. Five subpopulations of HDL were isolated from plasma and their chemical composition and cholesterol efflux capacity from human macrophagic THP-1 cells were evaluated

Materials and methods

Patients and study design

The study sample was composed by T2DM patients treated at the Lipid Clinic of the Instituto Nacional de Ciencias Médicas y Nutrición (INCMNSZ) in Mexico. Clinical phase was carried out at INCMNSZ, where the protocol was approved by the Ethics Institutional Committee in accordance with the Helsinki Declaration. Further analyses were performed at the INSERM Research Unit 1166 at the Hospital La Pitié - Salpétrière in France.

Males or postmenopausal women aged 20 to 65 years, free of major diabetes-related chronic complications and displaying HbA1c levels <8% were invited to participate. Patients had to be out of statin therapy for at least 24 weeks to be eligible for the study. Patients with previous diagnosis of elevated blood pressure had to be under a good control using anti-hypertensive drugs (blood pressure below 130/80 mmHg). Smoking patients were excluded. Patients with positive history of cardiovascular disease (CVD) or severe hyperlipidemia (defined as plasma total cholesterol > 300 mg/dL or triglycerides > 400 mg/dL) as well as secondary causes of dyslipidemia or conditions altering lipid profile (i.e.

liver diseases, infection with human immunodeficiency virus, rheumatologic diseases, and treatment with drugs that affect plasma lipid profile) were excluded. All patients signed an informed consent.

The sample size for the study was calculated assuming one group (one-sample comparison of mean) and 90% power to demonstrate a difference of 10% in the cholesterol efflux capacity of HDL as a result of the treatment. According to these criteria, 33 patients were required.

The study included four visits (at baseline and after 2, 4 and 10 weeks of the treatment). Three weeks before the baseline visit, an isocaloric dietary plan was prescribed involving 50%, 20% and 30% of energy uptake from carbohydrates, proteins and fats, respectively.

Patients arrived to the clinical center after a 10-12 hour fasting period and were instructed to take Atorvastatin 20 mg/day (one pill every night). Adherence to the therapy was measured on the following visits using three-day food records and pill counts. Anthropometric data were collected by a nutritionist. Body mass index (BMI) was calculated as weight (kg) divided by height (meters) squared. Glucose-lowering therapies and antihypertensive drugs remained constant during the study.

Laboratory measures

Blood samples were collected on every visit into EDTA-containing tubes as well as into tubes without anticoagulant. EDTA plasma and serum were separated using low-speed centrifugation, aliquoted and frozen at -70°C.

Lipid profile, levels of transaminases, insulin, apolipoproteins B and A-I, and core laboratory clinical chemistry were determined on every visit. HbA1c concentration was added to the evaluation at the first and the last visits. Plasma lipids and clinical chemistry parameters were measured using commercially available kits (Synchron CX5-delta®, Beckam Co®). Insulin was measured using an immunoenzymatic assay (Abbott®). Concentrations of apolipoprotein B and A-I were evaluated using immunonephelometric methods (Beckman®). Levels of glycated hemoglobin A1c were measured by HPLC (BioRad®). Non-HDL cholesterol was calculated by substracting HDL-C from total cholesterol. LDL-C was calculated by the Friedewald formula in subjects with triglycerides levels below 250 mg/dL.

Five subpopulations of HDL, specifically HDL2b, 2a, 3a, 3b and 3c, were isolated by single-spin isopycnic density gradient ultracentrifugation using a potassium bromide density gradient ultracentrifugal procedure for the isolation of the major lipoprotein classes from human serum.[49]**.** After isolation, HDL subpopulations were extensively dialyzed for 48 hours and stored at 4^{o}C for not longer than 5 days[50]. Total HDL from each subject was prepared by mixing all five individual HDL subfractions at their equivalent plasma concentrations.

Major chemical components of HDL (phospholipid, free cholesterol, total cholesterol, triglyceride and total protein) were measured in all subpopulations. Phospholipid, free cholesterol, total cholesterol and triglyceride were quantified using DiaSys® reagents. Esterified cholesterol was calculated as a difference between total and free cholesterol multiplied by 1.67 [49]. Total protein was determined using the BCA method (BioRad®). Total mass of each HDL subfraction was calculated as a sum of phospholipid, free

cholesterol, esterified cholesterol, triglyceride and total protein concentrations. Total HDL mass was calculated as the sum of total masses of five individual HDL subfractions.

The capacity to efflux cellular cholesterol was evaluated in HDL2b and 3c subpopulations representative of large, light HDL2 and small, dense HDL3, respectively, as well as in total HDL. Human monocyte-derived THP-1 cells were placed at $1x10^6$ cells/well in 24-well plates containing RPMI 1640, and PMA was added to differentiate the cells into macrophages [27]. After 48 hour incubation under CO_2, acetylated LDL was added to the wells at 50 µg/mL followed by the addition of 12.5 µL of [^{3}H]-cholesterol (1 µL of [^{3}H]-cholesterol/mL RPMI). After 48 hours, cells were washed with PBS twice and serum-free RPMI was added followed by HDL in PBS to a final concentration of 15 µg phospholipid/mL and final volume of 300 µL. Cholesterol efflux capacity of HDL particles was measured on the basis of their PL concentrations because PL was shown to represent the key component determining cholesterol efflux capacity of HDL [51]. After 4 hours, the supernatant was removed and radioactivity within the medium was determined by liquid scintillation counting. The cells were lysed, 500 µL of the isoprophanolol/hexane mixture was added to each well to extract lipids, and cholesterol content was measured using cholesterol reagent (DiaSys®); [^{3}H]-cholesterol was determined by scintillation counting following addition of scintillation liquid. The percentage of cholesterol efflux was calculated as (medium cpm) / (medium cpm + cell cpm) x 100%. Specific cholesterol efflux was determined by subtracting non-specific cholesterol efflux occurring in the absence of HDL. All measurements were done in triplicates.

Statistical analysis

194 Statistical analysis was performed using STATA 13 software package (StataCorp LP,
195 USA). Graphs were plotted using GraphPad Prism 5 software (2007 GraphPad Software
196 Inc)

197 The demographic, anthropometric and biochemical continuous variables are presented as
198 mean ± SD or median and interquartile range (25-75) after testing distributions for
199 normality, whereas categorical variables are presented as percentages. To calculate
200 significance of the treatment effects, paired t-test or sign-rank Wilcoxon-test for continuous
201 variables with normal or non-normal distribution were applied, respectively. McNemar X^2
202 test for categorical variables was used.

203 Cholesterol efflux and chemical components of HDL subpopulations are presented as
204 median and IQ range (10-90). To calculate significance of the effects of atorvastatin on
205 these variables, Wilcoxon signed-rank test was employed.

206 Significance level of $p \leq 0.05$ was considered significant, except for multiple comparisons
207 when the level of $p \leq 0.05/5 = 0.01$ was used according to Bonferroni's adjustment, in order
208 to account for multiple comparisons across five HDL subpopulations.

209 According to the response to the treatment, two subgroups were built selecting patients who
210 achieved 35% of reduction in apoB levels or not.

211 We estimated the level of sdLDL using the triglyceride/HDL-cholesterol ratio. Patients
212 with the ratio above 4 were assumed to display elevated levels of sdLDL. We generated
213 two subgroups according this criterion[52].

Finally, we did Spearman correlation r^2 tests between clinical and biochemical variables with cholesterol efflux to identified determinants of cholesterol efflux capacity of HDL. Regression models were done as another approach to identified determinants of cholesterol efflux.

Results

Seventy patients were recruited for the study. Ten patients were excluded for different reasons and 60 patients completed the protocol (*Figure 1)*.

The population predominately consisted of postmenopausal women (62%), with a mean age for all patients of 58±10 years (*Table 1*). Most (95%) of the patients were treated with metformin as a single therapy. Thirty percent of patients used metformin in combination with insulin or other glucose-lowering drugs, except thiazolidinediones. Thirty four percent were previously diagnosed with elevated blood pressure; all of them were well controlled (SBP, 127±16 mmHg and DBP, 78±10 mmHg). Former smoking was reported by 33% of subjects and the time between smoking withdrawal and the start of the study was at least one year. Patients maintained an isocaloric diet during the study (*Table 2*).

No significant effect of atorvastatin treatment on clinical parameters was observed (*Table 3*). The treatment resulted in significant changes in all lipid-related parameters assessed (Table 4). The treatment reduced plasma concentrations of total cholesterol (by -32%), LDL-C (by -50%), triglycerides (by -19%), and apoB (by -34%); all changes were significant (p<0.001). LDL-C or apoB levels did not change after the treatment in 6% of patients. There was a -4% (p=0.02) decrease in HDL-C levels. Finally, S-creatinine and

aspartate aminotransferase levels were decreased (by 5 and 4 %, p=0.005 and 0.02, respectively) by the treatment (*Table 4*).

Total mass of HDL subpopulations remained unchanged, except in HDL3a, with an increase from 65.7 to 69.2 mg/dL (p=0.01) after treatment. By contrast, atorvastatin significantly modified the concentrations of several components of the HDL subpopulations. Thus, free cholesterol in the small, dense HDL3c subpopulation was reduced from 0.29 (0.22-0.39) to 0.20 (0.15-0.28) mg/dL (p=0.0001). Triglycerides in the HDL3c subpopulation decreased from 0.49 (0.38-0.80) to 0.34 (0.20-0.56) mg/dL (p=0.0001). Total protein increased in the HDL3a subpopulation from 29.5 (27.2-35.1) to 32.3 (29.0-38.5) mg/dL, p=0.0008. (Figure 2-A, B and E, respectively). These changes conducted to some significant modifications in the proportional distributions of chemical components. The free cholesterol proportion in the HDL3c subpopulation decreased from 1.97 (1.4-2.4) to 1.5 (1.0-1.8)% ($p\leq$0.0001). Triglycerides in the HDL3c subpopulation decreased from 3.2 (2.3-4.8) to 2.4 (1.5 - 3.2)% (p<0.001). Phospholipids increased in the HDL2b subpopulation from 30.1 (27.3-33.3) to 31.3 (28.6-33.9)% (p=0.01) and decreased in HDL2a from 31.14 (29.3-33.3) to 30.8 (28.5-32.3)%, HDL3a from 28.2 (26.6-29.9) to 28.1 (26.3-29.2)% and HDL3b from 23.6 (21.5-25.7) to 22.8 (20.8-24.9)%, (p= 0.001, 0.01 and 0.003, respectively). Total protein increased in HDL3a from 46.7 (43.6-49.4) to 47.8 (44.8-49.2)%, HDL3b from 53. (50.5-58.5) to 54.8 (52.5-58.9)% and HDL3c from 63.2 (58.8-68.8) to 67.4 (63.1-70.2)%, (p=0.001, 0.01 and <0.001, respectively) (Figure 2-G, H, J, K).

By contrast, no effect of atorvastatin on cholesterol efflux capacity of either large, light HDL2b, small, dense HDL3c or total HDL was observed (*Figure 3*). No correlations were found between HDL2b, HDL3c and total HDL cholesterol efflux and clinical and

biochemical parameters after adjusting for glycemic control, statin response, sLDL, Apo B and triglyceride levels. As mentioned in Materials and methods, we used phospholipid concentrations to calculate the cholesterol efflux experiments, but we also recalculated it using total proteins, no changes in results were found (HDL 3c subfraction p= 0.07 and 2b subfraction p=0.39 and total HDL p= 0.20). We recalculated the cholesterol efflux taking baseline correlations of subpopulation components and cholesterol efflux showed a positive relationship between total protein content and cholesterol efflux capacity of HDL2b, Spearman Rho 0.31 (p=0.03). Regression models with cholesterol efflux capacity of HDL as dependent variable did not reveal significant determinants of the response to treatment. We did not find any correlations with changes in ApoB and composition or function of HDL.

Discussion

Our study did not find any differences in cellular cholesterol efflux towards large HDL2b and small HDL3c subpopulations, or towards total HDL after a short course of treatment with moderate doses of atorvastatin in patients with well controlled type 2 diabetes. Although simvastatin and fibrates have earlier been tested for their capacity to modify cholesterol efflux properties of HDL in patients with T2DM, this is the first study to evaluate the effect of atorvastatin on this functional metric in patients with T2DM. Furthermore, we report, for the first time, the effect of atorvastatin treatment on cholesterol efflux capacity of HDL subpopulations.

Previous studies showed a significant improvement on cholesterol efflux in HDL from THP-1 cells, after treatment with simvastatin and pitavastatin in men with type 2 diabetes

281 and in dyslipidemic patients, respectively. Both studies demonstrated a significant increase
282 in HDL-cholesterol concentrations and used ApoB-depleted serum as cholesterol acceptor.
283 As a consequence, the increase in HDL levels potentially explained the improvement in
284 cholesterol efflux capacity[44][45]. By contrast, we used HDL isolated from plasma by
285 ultracentrifugation rather apoB-depleted serum. Our technique adjusts for alterations in the
286 HDL concentration and phospholipid content, the latter providing a major contribution to
287 the efflux capacity [27]. The methodological differences in cholesterol efflux experiments
288 and the use of other type of statin can explain contradictions between our results and those
289 reported earlier [44][45].

290 Indeed, the different type and doses of statins can have differential effects on HDL function
291 and lipid metabolism[47]. There are two previous studies of atorvastatin on HDL function,
292 although not in patients with type 2 diabetes. One of them found that proteome of small
293 HDL3 of patients with cardiovascular arterial disease (CAD) was altered by the treatment
294 with atorvastatin after one year[53]. Rader et al. studied patients with preexisting CAD, and
295 in accordance with our findings, did not find modification of cholesterol efflux from J774
296 cells to apoB-depleted serum after 16 weeks of treatment with low (10mg) or high (80mg)
297 doses of atorvastatin, even after a modest increase in HDL-cholesterol concentrations[18].
298 These contradictory findings between studies on proteomics of HDL and cholesterol efflux
299 capacity after treatment with atorvastatin highlight the possibility that atorvastatin displays
300 a null effect on cholesterol efflux capacity but positive effects on other biological activities
301 where proteins play a pivotal role, for example its anti-oxidative capacity[46]. New studies
302 are necessary to elucidate this theory.

Importantly, our study included only patients with type 2 diabetes with moderately low concentrations of HDL cholesterol at baseline; this alteration is typical and part of the phenotype called “diabetic dyslipidemia”. Our results showed that after treatment, HDL-cholesterol levels decreased even more. We do not believe that the significant diminution of HDL concentration accounted for the lack of the effect of atorvastatin on cholesterol efflux capacity because, as mention earlier, our experiments adjusted for changes in the phospholipid content of HDL. Typically, statins display moderately positive effects on HDL-C levels; however, the effects of statins seem to be altered in patients with T2DM. In 2004, CARDS, a multicenter randomized placebo-controlled trial, showed a 9% reduction of HDL-C levels in patients with T2DM after 4 years of treatment with 10 mg of atorvastartin [54]. Chang (2013) reported a high prevalence of diminution of HDL-C (-3%) after one year of atorvastatin treatment in patients with T2DM [55]. Other clinical trials of statins provided similar findings. The mechanism of this response is not fully elucidated but can involve some enzymes and transfer protein, including lipoprotein lipase, hepatic lipase, and phospholipid transfer protein, involved in HDL metabolism and remodeling. Indeed, the function of these proteins has been reported to be impaired in an insulin-resistant milieu[2]. Additionally, it is known that the liver may represent the major source of cholesterol that circulates as HDL-C; prolonging the inhibition of HMG-CoA reductase by statins may, therefore, result in depletion of hepatic cholesterol, leading to decreased production of HDL-C[56].

Our studies have limitations. As we evaluated a short course of treatment with moderate doses of atorvastatin, we cannot rule out that higher doses or longer treatment modify the cholesterol efflux capacity of HDL. On the other hand, the strengths of the present study

include a higher number of patients in comparison with previous studies of statin effects on cholesterol efflux capacity of HDL [44] as well as homogeneity of patients. As to our knowledge, there is no other study with a similar design and technique to evaluate the cholesterol efflux capacity of HDL under statin treatment. The lack of effect of atorvastatin on cholesterol efflux is consistent with the residual risk observed in these patients even after they reach lipid goals with statins. Our results thereby suggest that some biological activities of HDL can be independent of the statins effect on ApoB or cholesterol levels.

In conclusion, our study showed that 10 weeks of treatment with a moderate 20 mg dose of atorvastatin does not modify the cholesterol efflux capacity of HDL particles in patients with well controlled type 2 diabetes. Due to the nature of our study, we cannot, however, translate these results into the commonly prescribed long-term statin therapy neither to other statin types.

Conflict of interest

The authors declared they do not have anything to disclose regarding conflict of interest with respect to this manuscript.

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Author contributions

All authors contributed equally in the design, development, results and discussion of the research.

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599 Tables and figures

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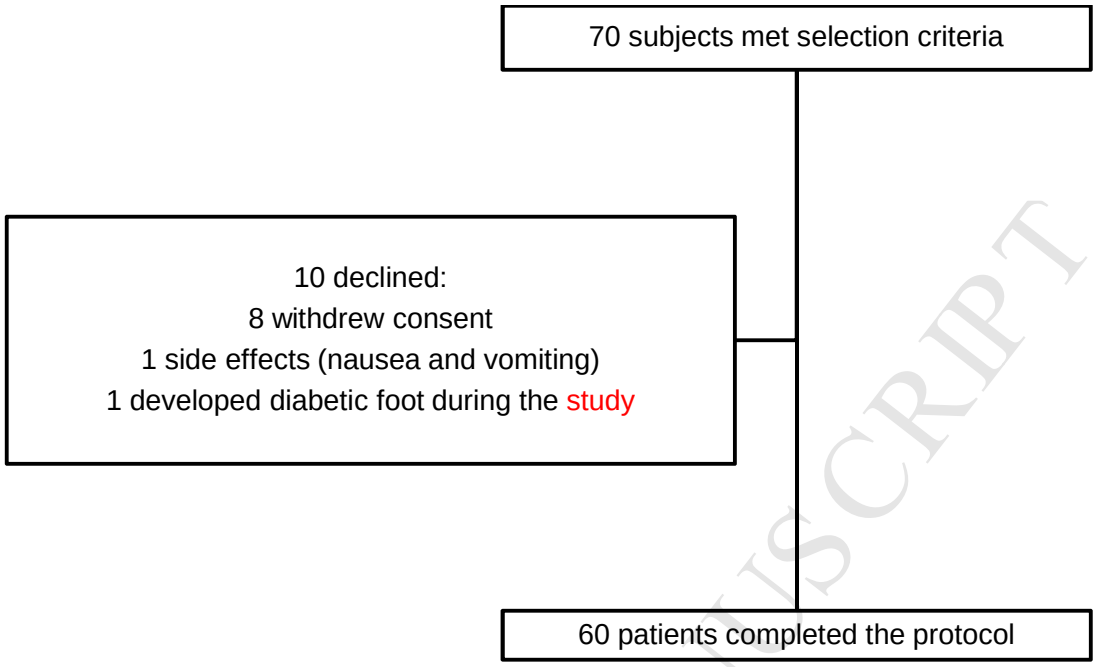

601

Figure 1. Study population, screening and follow-up.

602

603 Table 1 Clinical characteristics of the study population

N	60
Women/men %	62/38
Age yr	58 ± 10
BMI kg/m^2	28.6 ± 3.7
SBP mmHg	127 ± 16
DBP mmHg	78 ± 10
Age at diagnosis yr	50 ± 10
Diabetes evolution years	8 (4-12)
Metformin treatment %	95
Insulin treatment %	30
Other anti-diabetic drugs[a] %	25
Cardiovascular disease history %	39
Smoking history[b] %	21

604 N= 60. Data are presented as mean ± SD or median and interquartile

605 range (10-90) and as a percentage.

606 [a] Except thiazolidinediones

607 [b] At least one year after smoking withdrawal, Stata 13.

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609

610 Table 2 Energy intake through the study

	Before[a] treatment	After[a] treatment	*p* value
Energy intake kcal	1596 ± 439	1666 ± 438	0.21
Carbohydrates gr	206 ± 66	221 ± 62	0.10
Proteins gr	80 ± 19	83 ± 24	0.30
Fat gr	50 ± 21	50 ± 16	0.99
Fiber gr	27.4 ± 7.6	29.4 ± 8.3	0.09
Carbohydrates %	51 ± 6	53 ± 6	0.14
Proteins %	20 ± 2	19 ± 3	0.56
Fat %	28 ± 6	27 ± 5	0.23

611 [a]Data are presented as mean ± SD

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618 Table 3.- Clinical parameters before and after the atorvastatin treatment of patients with
619 T2DM

Characteristics	Before[a]	After[a]	*p* value
Weight kg (F/M)	66.2 (59.5-71.6)	65.7 (59.5-71.6)	0.27
	80.8 (74.5-86.7)	81.4 (73.8-86.0)	0.78
BMI Kg/m^2 (F/M)	29.1 (26.1-31.1)	28.4 (25.9-30.7)	0.29
	28.4 (26.7-32.5)	29.1 (25.8-32.9)	0.70
SBP mmHg	127 ±16	121 ±18	0.06
DPB mmHg	78 ±10	76 ± 9	0.09
Waist cm (F/M)	94.5 (88.5-99)/	91.8 (88.5-99.8)	0.08
	99.5 (95-104.2)	97.5 (95.104.5)	0.06

620 [a] Data are presented as mean ± SD or median and interquartile range (10-90)
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630 Table 4.- Serum biochemistry before and after the atorvastatin treatment of patients with
631 T2DM

	Before[a]	After[a]	Change %	*p* value
Total cholesterol mg/dL	184 ±32	127±27	-32	**>0.001**
LDL-cholesterol mg/dL	98 (82-117)	49 (39-62)	-50	**>0.001**
HDL-cholesterol mg/dL	47 (42-54)	45 (39-56)	-4	**0.02**
ApoB mg/dL	91 (82-120)	60 (49-76)	-34	**>0.001**
ApoA-I mg/dL	147 (129-163)	143 (124-165)	-3	**0.02**
Triglycerides mg/dL	154 (113-228)	125 (103-169)	-19	**>0.001**
Glucose mg/dL	125 (108-145)	127 (105-161)	+1.6	0.08
Insulin Ui/L	11.9 (8.4-18.1)	13.2 (9.8-19.3)	+11	0.16
HbA1c %	6.98 ±0.83	6.91 ±0.99	-2	0.89
Creatinine	0.72±0.16	0.69 ±0.16	-5	**0.005**
AST	24 (21-30)	23(21-28)	-4	**0.02**
ALT	23 (19-32)	25 (19-30)	+8	0.53
GGT	20 (15-27)	19 (15-26)	-5	0.11

632

633 [a]Data are presented as mean ± SD or median (interquartile range 10-90)

HbA1c, glycated hemoglobin ; apo B, apolipoprotein B; apo A-I, apolipoprotein A-I; AST, aspartate transaminase; ALT, alanine transaminase; GGT, gamma glutamil transaminase.

652

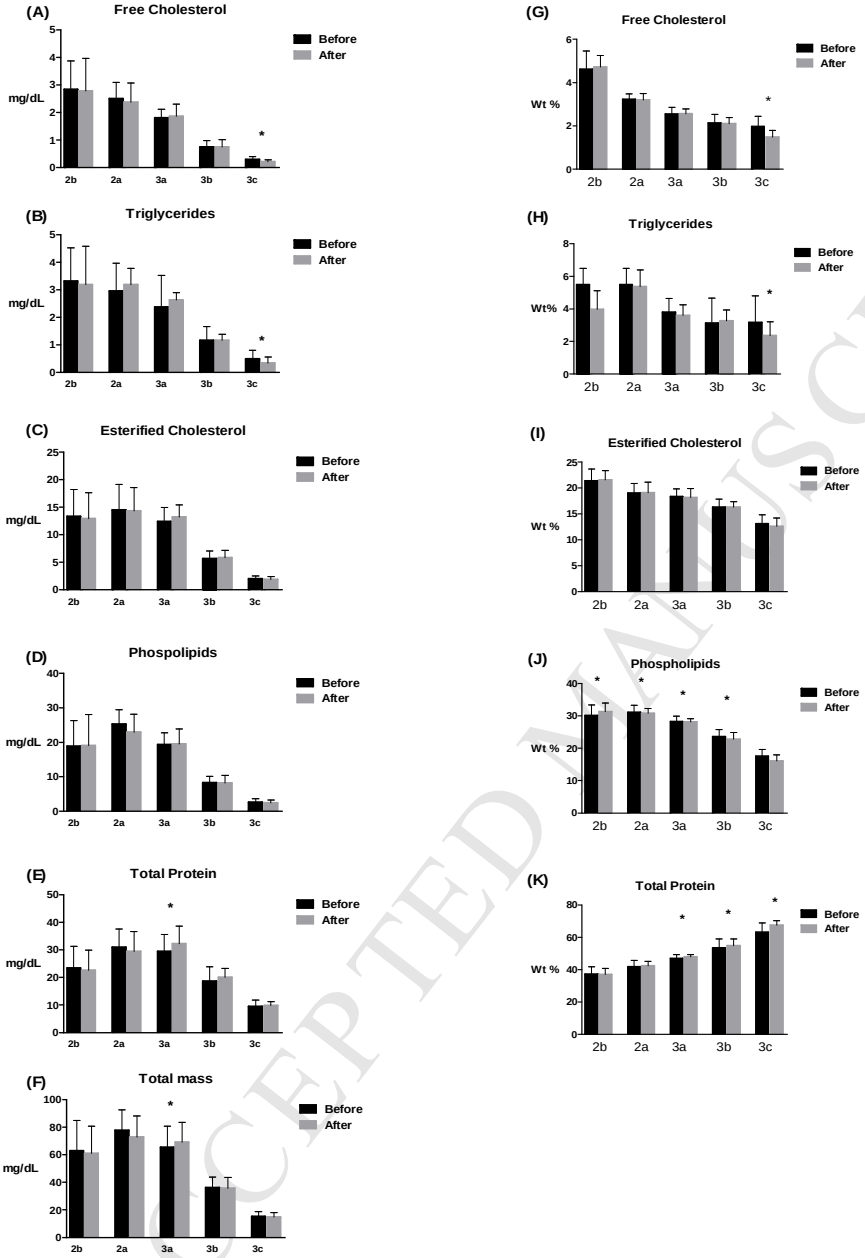
653 Table 5.- Cholesterol efflux capacity of HDL particles before and after the atorvastatin

654 treatment of patients with T2DM

Cholesterol efflux capacity	Before[a]	After[a]	*p* value
Total HDL %	3.78 (3-12-4.76)	3.59 (3.01-4.34)	0.32
HDL2b subpopulation %	2.86 (2.29-3.85)	2.93 (2.32-3.42)	0.84
HDL3c subpopulation %	6.89 (6.08-8.06)	6.66 (6.04-8.15)	0.88

655 [a]Data are presented as median (interquartile range 10-90)

656



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658 Figure 2. Total mass and chemical composition of HDL subpopulations expressed as
659 mg/dL (A-F) and as weight percentage of total mass (G-K) before and after atorvastatin
660 treatment in patients with T2DM.

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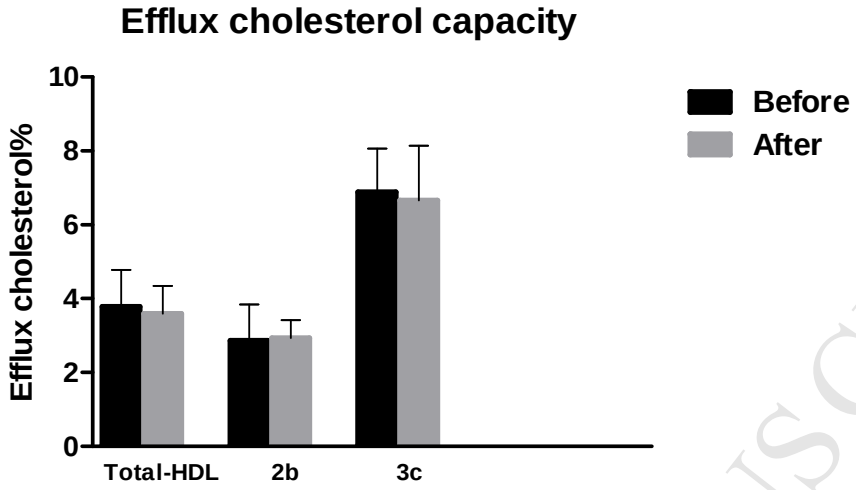

662

663 Figure 3.- Cholesterol efflux from THP-1 cells to total HDL and to HDL2b and 3c

664 subpopulations before and after atorvastatin treatment in patients with T2DM.

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Highlights

1. Cholesterol Efflux capacity of HDL is altered in subjects with type 2 diabetes
2. Low levels of HDL-Cholesterol and mild hypertriglyceridemia are typical in T2DM
3. Statin effects on HDL biological activities are contradictory across studies
4. Different classes of statins could have differentiated effects on HDL