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Does non-alcoholic fatty liver impair alterations of plasma lipoproteins and associated factors in metabolic syndrome?

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ABSTRACT

Background: Hepatic steatosis (HS) is closely associated to metabolic syndrome (MS). Both, VLDL-triglyceride oversecretion and intrahepatic deposits, can take place. We evaluated VLDL characteristics, CETP, hepatic lipase (HL), IDL and small dense LDL (sdLDL), in patients with HS associated to MS.
Methods: We studied 3 groups matched by age and sex: 25 MS patients with HS (diagnosed by ultrasonography), 25 MS patients without HS and 25 healthy controls. Main measurements were: lipid profile, free fatty acids, VLDL composition, VLDL size by HPLC, CETP and HL activities, IDL-cholesterol and sdLDL-cholesterol.
Results: Patients with HS presented higher triglyceride levels, HOMA-IR and free fatty acids, VLDL mass and VLDL-apoB ($p<0.05$). No differences in VLDL composition were observed. MS groups presented higher proportion of large VLDL than controls ($p<0.05$). HS group showed higher CETP than controls ($p=0.01$) and almost higher than MS without HS ($p=0.06$). CETP correlated with VLDL-cholesterol content, $r=0.48$, $p<0.005$. The increase in sdLDL-cholesterol correlated with CETP ($r=0.47$) and HL ($r=0.56$), independent of insulin resistance ($p<0.003$).
Conclusion: Despite intrahepatic fat, patients with HS secreted higher number of VLDL particles. CETP would have a remodeling action on VLDL in circulation, enriching it in cholesterol and also favoring, together with HL, the formation of sdLDL.

1. Introduction

Non-alcoholic fatty liver is considered the hepatic manifestation of metabolic syndrome (MS). In the last years, there has been evidence showing that non-alcoholic hepatic steatosis is an independent predictor of cardiovascular disease [1,2]. Possibly, one of the determinants of the increase in cardiovascular risk when hepatic steatosis (HS) is associated to MS would be a more atherogenic lipid–lipoprotein profile. There is already an overproduction of hepatic VLDL-triglycerides in MS, but otherwise, hepatic triglycerides deposition is induced [3,4]. Therefore, we considered it is important to know the characteristics of composition and size of the VLDL produced and secreted as a result of these apparently opposite mechanisms, since VLDL is the precursor lipoprotein of plasma lipid profile, it must be taken into account that lipoprotein alterations – regarding composition or structure – are associated with increased atherogenic potential [5,6].

In a previous study Adiels et al. described an overproduction of large VLDL rich in triglycerides associated with liver fat in type 2 diabetic men [7]. No references have been found so far in earlier stages of type 2 diabetes, like MS. In a recent publication, we studied a rat model of insulin resistance induced by diet, not yet diabetic, and we observed an increase in the secretion rate of VLDL rich in triglycerides and for sure larger, associated with intrahepatic fat accumulation, compared with control rats [8]. However, it should be noted that rats lack cholesteryl ester transfer protein (CETP), protein responsible for lipid molecule interchange between lipoproteins, therefore, inducing plasma particle composition remodeling [9,10]. Thus, in circulation particles are modified and this may be more pronounced if the activity of CETP is higher. There are no previous studies evaluating the activity of CETP and its relation to circulating VLDL type in patients with MS, with and without fatty liver.

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Another factor which regulates the concentration of the atherogenic lipoproteins, such as IDL and small dense LDL subfraction, is the hepatic lipase [11]. This enzyme could influence the plasma lipoprotein profile. Its activity in relation to non-alcoholic fatty liver and the lipid profile has not been studied yet. Our objective was to examine whether the plasma lipid–lipoprotein profile, including the characterization of VLDL and concentrations of IDL and small dense LDL, differ in patients with MS and presence of HS regarding to those with MS who do not present a HS, and also to evaluate in this context the possible role of CETP and hepatic lipase.

2. Materials and methods

2.1. Subjects

Patients were recruited from those who were attended at the Metabolism Section from the Endocrinology Division of the Durand Hospital, a general hospital located in the city of Buenos Aires, Argentina. From November 2007 to June 2009, patients with diagnosis of MS (ATPIII criteria) [12], were selected consecutively for the present study. The following exclusion criteria were considered: alcohol intake >20 g/day, type 2 diabetes, recent acute illness, drugs which could modify lipid levels or induce fatty liver [13], seropositive hepatitis B or C, or any other health problem which could lead to liver steatosis. After applying the exclusion criteria, all the MS patients were referred to the Hepatology Unit for ultrasound investigation of steatosis, performed by a single operator. According to ultrasound results, patients with MS were divided into 2 groups: with or without HS, depending on the presence or absence of "bright liver" as a characteristic echo pattern according to standard criteria (i.e. evidence of diffuse increase in echogenicity of the liver compared with that of the kidneys). A total of 71 patients were required to be attended in order to achieve the following matched groups: 25 patients with MS and hepatic steatosis matched by sex (18 females and 7 males) and age (±1 year) vs 25 MS patients with negative echogenicity evidence of HS. Due to the known high prevalence of fatty liver in MS, the total number of patients in each matched group selected for this study was limited and conditioned by the fact that it is less frequent to find patients with MS without HS.

In parallel, 25 clinically and biochemically healthy subjects who consumed <20 g alcohol/day were recruited among hospital employees as control group. They underwent hepatic ultrasonography in order to discard steatosis and finally were selected taking into account the gender and age matching with the MS groups. Waist circumference, weight and height of each participant were measured and BMI calculated. Written informed consent was required of all the participants to be included in the study and for the acceptance of heparin administration necessary to hepatic lipase determination. The heparin administered for the release of hepatic lipase from the endothelium surface is used in a very low dose and no adverse effects were reported**.** The study had the approval of the Ethics Committee of Durand Hospital and of the Faculty of Pharmacy and Biochemistry, University of Buenos Aires.

2.2. Samples

After a 12-h overnight fast blood samples were drawn. Serum or plasma was kept at 4 °C within 48 h for the evaluation of liver enzymes, glucose, lipids and lipoproteins, or stored at −70 °C for further determination of insulin, free fatty acids, CETP, small dense LDL and VLDL and IDL isolation by ultracentrifugation. Heparin (60 UI/kg body weight) was administered intravenously for the determination of hepatic lipase activity in those participants who accepted the administration. Ten minutes after, blood was drawn from the contralateral arm and the postheparin plasma was kept at −70 °C until its processing.

2.3. Biochemical determinations

Aspartate-aminotransferase, alanine-aminotranferase and γ-glutamyltranspeptidase, total cholesterol, triglycerides and glucose were measured in serum using commercial kits (Roche Diagnostics, Mannheim, Germany) in a Hitachi 917 autoanalyzer, intra-assay CV<1.9%, inter-assay CV<2.4%, averaging CV values of these parameters. HDL and LDL cholesterol were determined by standardized selective precipitation methods [14,15] intra-assay CV<2.0% and inter-assay CV<3.0%. Apo A-I and apo B were determined by immunoturbidimetry (Roche Diagnostics, Mannheim, Germany), intra-assay CV<1.9% and inter-assay CV<2.5%, for both parameters. Free fatty acids were determined by a spectrophotometric method (Randox, UK), intra-assay CV<2.6% and inter-assay CV<3.9% and insulin was measured with Immulite/Immulite 1000 Insulin (Siemens, Tarrytown, NY). In order to estimate insulin resistance, the HOMA index was calculated [16].

2.3.1. CETP activity

Cholesteryl ester transfer protein activity was determined in serum samples as described previously [17]. Briefly, it is utilized for the evaluation of the ability of serum to promote the transfer of tritiated cholesteryl esters from a tracer amount of biosynthetically labeled HDL3 (3H–CE–HDL3) (NENLife Science Products, Boston MA) towards serum apo B-containing lipoproteins. Results were expressed as the percentage of 3H-cholesteryl esters transferred from HDL3 to apo B-containing lipoproteins, per ml, per h. Measurements were all carried out in duplicate within the same assay. The intra-assay CV was 4.9%.

2.3.2. Hepatic lipase activity

Hepatic lipase activity in postheparin plasma was determined by measuring the oleic acid produced by the enzyme-catalyzed hydrolysis of an emulsion containing [3H]-triolein (Amersham TRA 191; Amersham, Buckinghamshire, UK) as previously published [18]. Results were expressed as μmol/ml h of free fatty acids of postheparin plasma. Using triplicate analysis, the intra-assay CV was 4% and the inter-assay CV 9%.

2.3.3. Lipoprotein measurements

2.3.3.1. VLDL and IDL isolation and analysis. Lipoproteins were isolated by sequential preparative ultracentrifugation [19], VLDL [density (d)< 1.006 g/ml] and IDL [d:1.006–1.019 g/ml], in a Beckman XL-90 using a fixed-angle rotor type 90 Ti. Each run was performed at 105,000×g, for 18 h, at 14 °C. Purity of lipoprotein was tested by agarose gel electrophoresis. Isolated VLDL composition was characterized by the following parameters: cholesterol, triglycerides and apo B, using the methods previously described, phospholipids [20] and proteins by the Lowry method. Data was expressed as the percentage of each component, and their sum as circulating VLDL total mass per plasma decilitre. In order to assess IDL concentration, its cholesterol content was measured, as described before.

2.3.3.2. VLDL size by high performance liquid chromatography (HPLC). High performance liquid chromatography (HPLC) with size exclusion columns is an alternative method for classifying and quantifying lipoproteins on the basis of particle size. In brief, isolated VLDL were injected in a column TSK-Gel Lipopropack XL, 7.8 mm ID×30 cm (Tosoh, Japan) and runs were performed using as mobile phase: Tris acetate buffer 0.05 mol/l (pH 8) containing 0.3 mol/l sodium acetate, 0.05% sodium azide and 0.005% Brij-35. Flow rate was 0.5 ml/min and the column eluate was monitored at 280 nm [21]. For the conversion of elution time in particle diameter, a standard curve was used, constructed with the logarithm of retention time and the logarithm of the diameter of standard diameter latex particles, 100 nm in diameter (Fluka, Sigma-Aldrich) and of 27 and 39 nm in diameter (Magsphere INC).

According to Okazaki et al., large VLDL was considered between 45 and 80 nm [22]. Data is expressed as the percentage of the peak area corresponding to large VLDL, using the ChromQuest 4.1 integration program.

2.3.4. Small dense LDL cholesterol

The small dense LDL subfraction was measured by a precipitation commercial kit (sdLDL-C, Denka Seiken, Japan) using heparin sodium salt and $MgCl_2$. After precipitation LDL-cholesterol was measured by a direct and selective homogenous assay method (LDL-EX; Denka Seiken, Japan) [23]. Results are expressed in mg of small dense LDL cholesterol/dl.

2.4. Statistical analysis

Data is presented as means ± SD or median (range) according to normal or skewed distribution. A $p<0.05$ was considered significant. One-way analysis of variance (ANOVA) or Kruskal–Wallis ANOVA was used to test differences among groups. Further evaluation was performed using Tukey multiple comparison test. Pearson or Spearman analysis, for parametric or non parametric variables, was used to determine correlations between parameters. A multivariate stepwise regression model was developed in order to assess associations between parameters. All analyses were performed using SPSS 11.5 software (Chicago, IL).

3. Results

Table 1 shows patients' and controls' characteristics. Both MS patients showed an increased BMI in comparison to controls, showing a tendency to an increase in MS patients with HS when compared to MS patients without HS ($p=0.07$). As regards to waist circumference, MS patients with HS did show an increase in comparison to those MS patients without HS, $p=0.034$.

Patients with HS presented the highest glucose and insulin plasma levels and HOMA-IR (Table 1). According to the clinical situation, liver enzyme – especially alanine-aminotranferase and γ-glutamyltranspeptidase –, were significantly increased in group with HS vs without HS or controls, $p<0.05$. However, just 4 out of 25 HS patients presented alanine-aminotranferase elevation between 1.5 and 4 times the upper normal values.

Table 1
Clinical, laboratory characteristics and lipids and lipoprotein profile of the subjects studied, divided into healthy controls and metabolic syndrome (MS), with and without hepatic steatosis (HS).

	Healthy Controls	MS without HS	MS with HS
n	25	25	25
Age (years)	44 ± 16	46 ± 16	46 ± 17
BMI (kg/m^2)	24.1 ± 4.3 (*)	34.4 ± 5.8 (†)	36.0 ± 6.0 (†)
Waist (cm)	80 ± 12 (*)	103 ± 8 (†)	109 ± 9 (‡)
Insulin (μU/ml)	5.8 (2-19.2) (*)	10.9 (3.3–35.4) (†)	15.7 (5.9–70) (‡)
Glucose (mg/dl)	86 ± 8 (*)	98 ± 9 (†)	109 ± 8 (‡)
HOMA-IR	1.8 ± 1.2 (*)	3.3 ± 2.0 (†)	5.8 ± 4.4 (‡)
AST (U/l)	22 ± 9	24 ± 14	33 ± 20
ALT (U/l)	21 ± 9 (*)	25 ± 16 (*)	45 ± 32 (†)
ALT/AST	0.9 ± 0.2 (*)	1.0 ± 0.3 (*)	1.4 ± 0.3 (†)
GGT (U/l)	19 ± 15 (*)	26 ± 17 (*)	53 ± 44 (†)
Total cholesterol (mg/dl)	201 ± 43	207 ± 42	221 ± 40
LDL cholesterol (mg/dl)	128 ± 49	150 ± 45	158 ± 43
HDL cholesterol (mg/dl)	56 ± 17 (*)	41 ± 8 (†)	39 ± 8 (†)
Triglycerides (mg/dl)	90 ± 34 (*)	157 ± 58 (†)	213 ± 118 (‡)
Free fatty acids (mmol/l)	0.43 ± 0.16 (*)	0.49 ± 0.19 (*)	0.64 ± 0.29 (†)
Apo AI (mg/dl)	162 ± 38	138 ± 21	140 ± 24
Apo B (mg/dl)	105 ± 30	115 ± 31	116 ± 36
IDL cholesterol (mg/dl)	5.3 ± 1.8	5.7 ± 2.8	4.6 ± 1.7
sdLDL cholesterol (mg/dl)	9.1 ± 7.9 (*)	27.4 ± 16.6 (†)	29.9 ± 15.3 (†)

Data is mean ± SD or median (range) for insulin which is skewed distributed. (*) (†) (‡): different symbols indicate statistical differences between groups, $p<0.05$ (ANOVA), similar symbols indicate no differences between groups. AST: aspartate aminotransferase; ALT: alanine-aminotranferase; BMI: body mass index; GGT: gamma-glutamyltranspeptidase; HOMA-IR: homeostasis model assessment for insulin-resistance index; sdLDL: Small dense LDL.

Lipid–lipoprotein profile is also observed in Table 1. Even though no differences were observed in total or in LDL cholesterol, HDL cholesterol was lower in both MS groups compared to controls. Triglycerides were significantly different in all groups, $p<0.035$. It is worthy to note that free fatty acids levels increase significantly in the group with HS when compared to the other groups, $p<0.030$. No differences in IDL cholesterol, apo A-I and apo B concentrations were obtained.

When VLDL was isolated, an increase in VLDL mass and VLDL apo B was observed in patients with HS, in comparison to those without HS and controls (Table 2). These results would indicate a higher number of circulating VLDL particles in MS associated with HS. However, no differences between groups were noted in percentage chemical composition of the total VLDL fraction (Table 2). VLDL size measurements by HPLC revealed the following proportions of large VLDL particles: median (range), MS with HS: 16.0% (2.9–33.9); MS without HS: 17.6% (1.3–57.4) and controls: 6.3% (0–21.0). Both MS groups presented higher proportion of large VLDL in comparison to controls, $p<0.05$. After applying linear regression analysis considering the three groups, it is important to mention significant correlations between free fatty acids, not only with VLDL-triglycerides mg/dl ($r=0.39$, $p=0.03$) but also with the percentage of large VLDL ($r=0.47$, $p=0.01$), even after adjusting by HOMA-IR, F: 4.62 and F: 5.89 respectively, $p<0.04$.

Other factors associated to lipoproteins metabolism, such as CETP and hepatic lipase were evaluated. Fig. 1-A shows that patients with HS present a significant increase of CETP activity in comparison to controls, $p=0.014$, and a strong tendency to increase when compared to patients without HS, $p=0.06$. As CETP has a remodeling action on lipoproteins composition, it was correlated in the whole population with VLDL-triglyceride% and VLDL-cholesterol%, finding that the latter was associated to CETP, ($r=0.48$; $p<0.005$), Fig. 2. After adjustment by HOMA-IR, BMI and waist circumference, correlation was still significant, $F=6.07$; $p=0.02$. Although CETP did not correlate with large VLDL proportion in the whole population ($r=0.03$, $p=0.87$) a tendency to correlate inversely was found when only MS groups were considered ($r=-0.51$, $p=0.06$).

Fig. 1-B indicates that there were no differences in hepatic lipase activities between groups, $p>0.283$. However, hepatic lipase activity significantly correlated with IDL cholesterol, ($r=-0.57$; $p<0.002$) according to its well-known enzymatic role on IDL as substrate.

Finally, small dense LDL concentration was assessed to complete the lipoprotein profile in each group. As expected, both MS groups, with and without HS, presented higher small dense LDL cholesterol than controls: $p=0.01$ and $p=0.03$ respectively, but HS group did not show differences against MS without fatty liver (Table 1). Plasma concentration of small dense LDL cholesterol significantly correlated with CETP ($r=0.47$; $p<0.003$) and hepatic lipase ($r=0.56$; $p<0.002$) activities.

4. Discussion

This study was carried out to attempt to elucidate whether plasma lipid–lipoprotein profile, associated factors and specially VLDL characteristics, differ in patients with MS associated with non-alcoholic HS, in comparison to MS patients without HS and healthy controls. The results could partly explain the increased risk in cardiovascular disease in non-alcoholic fatty liver disease [24,25]. Patients with associated fatty liver were more insulin-resistant, showed increased free fatty acids, and mainly presented higher VLDL mass and VLDL-apo B, indicating an increase in the number of particles. Both MS groups showed more predominance of large VLDL

Table 2
VLDL in the subjects studied, divided into healthy controls and metabolic syndrome (MS), with and without hepatic steatosis (HS): total mass, VLDL-apoB and total fraction chemical composition.

	VLDL total mass (mg/dl)	VLDL Apo B (mg/dl)	Percentage composition			
			TG (%)	Cholesterol (%)	Proteins (%)	Phospholipids (%)
Healthy controls (n = 25)	58.5 ± 39.2 (*)	3.7 ± 2.0 (*)	52.7 ± 8.1	16.8 ± 4.2	11.6 ± 7.0	18.9 ± 5.8
MS without HS (n = 25)	64.6 ± 50.2 (*)	3.7 ± 1.7 (*)	49.4 ± 10.3	17.4 ± 4.6	11.2 ± 5.1	22.0 ± 8.7
MS with HS (n = 25)	120.2 ± 108.0 (†)	5.8 ± 3.6 (†)	52.1 ± 12.3	16.2 ± 5.3	12.3 ± 6.1	19.4 ± 11.0

Data is mean ± SD (*) (†): different symbols indicate statistical differences between groups, $p<0.05$ (ANOVA), similar symbols indicate no differences between groups. TG: triglycerides.

than controls and no differences in circulating VLDL composition. However, patients with HS tended to show increased CETP activity, which correlated directly with VLDL-cholesterol content, suggesting that in circulation, VLDL suffers from more alterations. No differences in hepatic lipase activity were observed but the correlations obtained show that the higher the enzyme activity, the greater the formation of small dense LDL, regardless of the insulin-resistance degree.

The selection, matched by age and sex, of patients with MS divided into those with presence and absence of HS, was considered an adequate design to achieve the stated aim, being fatty liver the only criterion of subdivision.

Ultrasonography has demonstrated to be a useful tool for the detection of HS, being an inexpensive and non-invasive method with a high diagnostic accuracy, as was demonstrated by several authors [26,27]. A limitation of this study is that liver biopsies, considered the gold standard for the diagnosis of hepatic steatosis, were not available in these subjects. Based on aminotransferases values, only a moderate increase in alanine-aminotranferase was observed in patients with HS, suggesting that they may not present steatohepatitis. However, histological studies are necessary for the detection of this stage of the disease. The higher values of γ-glutamyltransferase in patients with HS should be taken into account because of its recent association to cardiovascular risk [28].

The higher insulin-resistance degree found in HS group determines an increase in free fatty acids that promotes their influx into the liver and would lead to triglyceride overproduction and deposit [29]. Moreover, an increase in the triglyceride secretion rate is likely to occur, evidenced by the higher triglyceride concentration in the HS group despite the fat deposits and taking into account that, under these conditions, catabolic rate would not be impaired [30]. We have recently observed an over VLDL secretion rate in an insulin-resistance rat model presenting fatty liver in comparison to controls [8]. The increase in VLDL mass and number of particles found in patients with HS also supports this concept.

Herein, VLDL chemical composition and size were assessed considering that it is unclear whether the apparently opposite mechanisms, increased VLDL secretion on the one hand and hepatic triglyceride

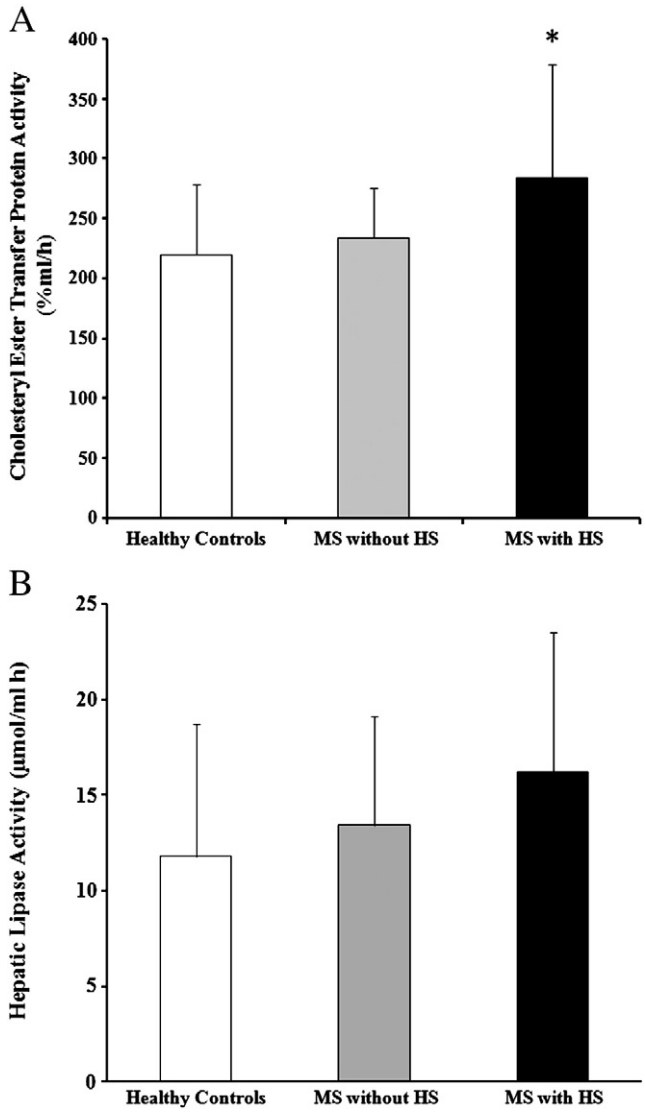

Fig. 1. A — Bar graphs show CETP activity in the three studied groups, healthy controls [n = *24*] (white bar), metabolic syndrome (MS) without hepatic steatosis (HS) [n = *23*] (grey bar) and MS with HS [n = *24*] (black bar). * indicates, $p = 0.01$ and $p = 0.06$ when compared with healthy controls and MS without HS, respectively. B — Bar graphs show hepatic lipase activity in the three studied groups, healthy controls [n = *25*] (white bar), MS without HS [n = *25*] (grey bar) and MS with HS [n = *25*] (black bar). No significant differences were found among groups.

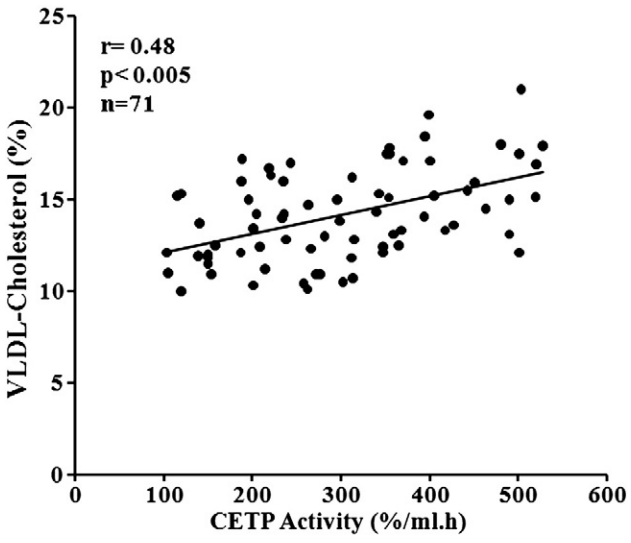

Fig. 2. correlations between CETP activity and VLDL-cholesterol percentage.

deposits on the other, impact on circulating VLDL characteristics. No differences in percentage chemical composition, in total VLDL fraction, were found among groups. This study is limited by the fact that we analyzed the total heterogeneous VLDL fraction in each patient without identifying subfractions or performing *in vivo* kinetic studies. From Kelley et al.'s study, it can be deduced that type 2 diabetic patients with HS, compared with those without HS, showed higher VLDL-triglyceride concentration assessed by nuclear magnetic resonance. However, there are no comments about whether this could be due to an increase in the number of particles or an increase in triglyceride content per VLDL particle [31]. More recently, Toledo et al. observed large VLDL triglyceride enriched in type 2 diabetic patients subdivided by the presence and absence of HS. Moreover, they did not find differences in the number of particles between groups [32]. As far as we know, all evidence arises from studies carried out on type 2 diabetic patients and not in previous stages such as metabolic syndrome without diabetes.

VLDL size was assessed by HPLC because of its short experimental time, small sample requirement, high resolution and excellent reproducibility. There was a predominance of large VLDL particles in both MS groups compared to controls. The secretion of large VLDL particles associated with liver fat content in type 2 diabetic patients in comparison to healthy controls has already been described [7]. However, no differences in large VLDL proportion were found between MS groups, with and without HS. Therefore, based on these results, predominance of large VLDL particles would not be affected by fatty liver.

One of the main results of the present study was the CETP activity, because there is no previous data regarding CETP in this context. Patients with HS showed higher CETP activity when compared to controls and a tendency to an increase ($p = 0.06$) regarding those MS patients without HS. It is important to highlight the positive correlations between CETP and VLDL-cholesterol, and negative with large VLDL proportion, even after adjusting by HOMA-IR. Even though, we have previously described VLDL rich in triglycerides associated to fatty liver in an insulin-resistant rat model [8], it is important to bear in mind that rats lack CETP [9]. Therefore, our results carried out on humans suggest that in HS associated to insulin resistance, VLDL would be secreted rich in triglycerides and then, in circulation, suffer from an interchange of triglycerides by cholesterol, mediated by CETP. This mechanism may explain the reasons why we did not find circulating VLDL rich in triglycerides in patients with HS associated to MS. Further experimental data, for example from incubating isolated VLDL with CETP, would be necessary to confirm this mechanism.

Although the presence of postprandial remnants in patients with HS associated to MS has already been described [33], in our study IDL cholesterol did not show an increase in its fasting levels in any of the groups. We had expected higher IDL levels in MS patients; however the finding was in concordance with Toledo et al.'s results, who reported that IDL was not influenced by the presence of HS [32]. The inverse correlation between IDL cholesterol and hepatic lipase activity confirms the role of the enzyme in hydrolyzing IDL triglycerides.

Small dense LDL is the last step of the delipidation chain, and has been proposed as an insulin-resistance marker by many authors [34]. As expected, both groups with MS presented higher levels of small dense LDL in comparison to healthy subjects, but without differences between them. A remarkable fact is that both, hepatic lipase and CETP have a crucial role in the production of small dense LDL particles, which was supported by the significant positive correlations between these factors and small dense LDL.

A limitation of this study was the small number of subjects included in each group since that it was conditioned by the restricted number of MS patients without HS; in spite of this, clear results were observed leading to further studies with greater number of patients.

Finally, this study provides clinical data in order to elucidate whether non-alcoholic fatty liver associated to MS impairs alterations of plasma lipoproteins and associated factors contributing to the atherogenic profile.

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