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The Role of Triacylglycerol in the Oxidizability of Low Density Lipoproteins and High Density Lipoproteins: Possible Contributions to Atherosclerosis in Metabolic Syndrome

Roberta Cazzola[1]*, Gabriele Piuri[1], Micaela Garziano[1], Erica Cassani[2], Anna Boggio[2], Michela Barichella[2], Fulvio Muzio[2] and Benvenuto Cestaro[1]

[1]*Deptartment of Biomedical and Clinical Sciences "L. Sacco", University of Milan, Milan, Italy*

[2]*Clinical Nutrition and Dietetic Unit, Parkinson Institute, Milan, Italy*

***Corresponding author:** Roberta Cazzola, Department of Biomedical and Clinical Sciences "L. Sacco", University of Milan, Italy. Tel: +39-0250319690; Fax: +39-0250319697; E-mail: roberta.cazzola@unimi.it

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Abstract

Objective: Metabolic Syndrome (MetS) is a cluster of Cardio-Vascular Disease (CDV) risk factors including visceral obesity, hyperglycemia, hypertension, low HDL cholesterol, hypertriglyceridemia and increased oxidative stress. Pyrene-lipid derivatives selectively incorporated into the hydrophobic core and surrounding amphipathic envelope of LDLs and HDLs are useful markers of the oxidizability of these lipoprotein regions. We performed an observational study aimed at investigating the effect of lipid composition, in particular triacylglycerol (TAG) levels, on the oxidizability of the core and the envelope of LDLs and HDLs in MetS.

Methods: We induced changes in the chemical composition of lipoprotein *in vivo* by placing on a hypocaloric balanced diet fifteen overweight and moderately obese men (BMI: 25-35 kg/mq) with MetS until they lost at least 5% of their initial weight. The core and the surface of LDLs and HDLs were labeled with selective pyrenic probes. Susceptibility to 2,2'-azobis-2-methyl-propanimidamide-dihydrochloride-induced peroxidation was measured following kinetically the decrease of fluorescence of these probes. The length of the lag phase (lag-time) of peroxidation kinetic were calculated and used as indices of lipoprotein oxidizability. In addition, we measured paraoxonase activity and plasma oxidative status.

Results: After weight loss, together with a reduction of triglyceridemia and the improvement of the other CDV risk factors associated with MetS, we observed a massive transfer of TAG from HDLs toward LDLs. In the LDLs core, the increased TAG concentration halved the resistance to peroxidation, while in the HDLs core the reduction of this parameter doubled the value of lag-time. In the lipoprotein hydrophobic cores, the duration of lag-time correlated directly with ratio between cholesteryl esters and TAG. No significant changes were found in paraoxonase activity and plasma oxidative status.

Conclusions: Less oxidizable HDLs, but also more oxidizable LDLs seem to accompany the improvement of different metabolic factors induced by weight loss in obese men with MetS.

Keywords: Triacylglycerol; Metabolic syndrome; LDL; HDL; Lipoprotein oxidation; Caloric restriction; Weight loss

Abbreviations AAPH: 2,2′-azobis (2-methylpropionamidine) dihydrochloride; BMI: Body Mass Index; CE: Cholesteryl Esters; CETP: Cholesterol Ester Transfer Protein; CRP: C-reactive Protein, DCF-DA: Dichlorofluorescin-diacetate; DTPA: Diethylene Triamine Pentaacetic Acid; HOMA-IR: Homeostatic Model of Assessment for Insulin Resistance; LCAT: Lecithin-Cholesterol Acyltransferase; LOOH: Lipid Peroxides; MDA: Malondialdehyde; MetS: Metabolic Syndrome; PyrPC: β-(pyren-1-yl) Decanoyl γ-palmitoyl L-α Phosphatidylcholine; PyrCE: Cholesteryl (pyren-1-yl) Hexanoate; PON-1: Paraoxonase; PUFA: Polyunsaturated Fatty Acids; PL: Phospholipids; ROS: Reactive Oxygen Species; TAG: Triacylglycerols; TRAP: Total Peroxyl Radical-trapping Potential

Introduction

The oxidation of Low-Density Lipoproteins (LDLs) is recognized as one of the major trigger elements of atherosclerosis progression. This process is regulated by several factors, such as the concentration of Reactive Oxygen Species (ROS) released by the stressed tissues, in particular vascular endothelium, the oxidizability of LDLs, and the protective effect of High-Density Lipoproteins (HDLs). Reverse cholesterol transport from the peripheral tissues is the best-known and salient activity of HDLs; however, they have other potential cardio-protective actions. In fact, mainly because of their antioxidant and anti-inflammatory properties, HDLs prevent the formation of oxidized LDLs and contribute to the neutralization of their effects [1,2], remove lipid hydroperoxides from tissues [3,4], and inhibit both platelet aggregation [5] and endothelial cell adhesion molecule expression [6]. Moreover, paraoxonase-1 (PON-1), an aryldialkylphosphatase capable of hydrolyzing a wide variety of substrates including toxic oxidized lipids, circulate associated to HDLs. PON-1 activity can vary by over 40 fold between individuals, mainly because of genetic polymorphisms

[7]; however, its enzymatic activity is modulated also by dietary factors, in particular lipids, and is inhibited by oxidized lipids [8]. Oxidative modification and changes in the chemical composition can reduce the functionality of HDLs and their associated enzymes [9-11].

Lipoproteins are formed by a core of hydrophobic lipids surrounded by an envelope of amphipathic lipids and proteins, and each of these components contributes to determine lipoprotein susceptibility to oxidation. The relationships between the lipid components of LDLs (especially fatty acids and cholesterol) and their susceptibility to oxidation has been widely investigated [12-14], while such information on HDLs is scarce. However, even if TAG are considered an important biomarker of cardiovascular disease risk because of their association with atherogenic remnant particles and apo C-III (a pro-inflammatory and pro-atherogenic apolipoprotein) [15], a direct role of these compounds in the atherogenic process has not yet been established. In addition, lipoprotein oxidizability has been principally investigated considering the entire lipoprotein particle. Nevertheless, we have previously shown that pyrene-lipid derivatives selectively incorporated into the hydrophobic core and surrounding amphipathic envelope of LDLs and HDLs are useful markers of the oxidizability of the region in which they are localized [16]. In addition, using pyrene labelled HDLs, we have also demonstrated that a higher ratio between the TAG and Cholesteryl Esters (CE) dramatically increased the fluidity and the oxidizability of hydrophobic core of HDLs in mixed dyslipidemic men [17].

Metabolic Syndrome (MetS) is a condition associated with obesity, especially abdominal obesity, characterized by increased oxidative stress, hypertriglyceridemia and low levels of HDL-cholesterol together with abnormal glucose tolerance and hypertension. MetS dyslipidemia is promoted by an impaired cellular response to insulin stimulation (insulin resistance) of target tissues, primarily liver, muscle, and adipose tissue. All the conditions that characterize MetS are among the leading causes of deaths worldwide, in addition MetS increases the risk of type 2 diabetes mellitus fivefold and cardiovascular disease threefold [18]. MetS hypertriglyceridemia is characterized by an increase of TAG in all lipoprotein classes [19]. An augmented ratio between TAG and CE in HDLs hydrophobic core contributes to a great clearance of this class of lipoproteins by the kidney [19], and, as mentioned above, increases the susceptibility to oxidation and changes the fluidity status of the hydrophobic core of these particles [17]. However, to the best of our knowledge, the role of TAG in the oxidizability of LDLs and HDLs hydrophobic cores in MetS has not yet been thoroughly investigated. Lipid composition of lipoproteins is influenced by genetic factors, disease conditions and lifestyle. It can be modified ex-*in vivo* by incubating plasma with different lipids or *in vivo* by changes in life style and/or weight loss. Moreover, intentional weight loss can improve dyslipidemia and oxidative stress and these metabolic benefits are often found after modest weight loss (5% of initial weight) [20].

We performed a pilot study with the aim to investigate the effect of lipid composition, in particular of TAG levels, on the oxidizability of LDLs and HDLs in MetS. To this purpose, we induced changes in the chemical composition of lipoprotein *in vivo* by placing on a hypocaloric balanced diet obese men with MetS until they had lost at least 5% of their initial weight, and we studied the effects of these changes on the oxidizability of the hydrophobic core and the surrounding amphipathic surface of LDLs and HDLs. In addition, we investigated also the effects of diet-induced weight loss on PON-1 activity and plasma oxidative status.

Materials and Methods

Materials

Organic solvent (analytical grade) and the other chemicals were purchased from Sigma-Aldrich (Milan, Italy) with the exception of the d-ROMs kit that was purchased from Diacron International (Grosseto, Italy).

Subjects

The subjects included in the present study were originally part of the "*Oxidative Stress, Inflammation, and Lipoprotein in Metabolic Syndrome*" study (ClinicalTrials.gov Identifier: NCT03553381). From this cohort we defined and selected 15 overweight and moderately obese Caucasian men (25 kg/m^2<BMI<35 kg/m^2) who on enrollment had metabolic syndrome defined according to International Diabetes Federation (i.e., waist circumference>94 cm plus any two of the following four components: 1) triglyceride level ≥ 150 mg/dL^2); 2) HDL cholesterol<40 mg/dL; 3) raised Blood Pressure (BP): systolic BP ≥ 130 or diastolic BP ≥ 85 mmHg or antihypertensive medication; 4) fasting plasma glucose ≥ 100 mg/dL) [21] and who after following a Mediterranean-style balanced hypo-caloric diet had lost at least 5% of their initial weight. The study was approved by the ethics committees of the Istituti Clinici di Perfezionamento of Milan and of L. Sacco Hospital of Milan and was carried out in accordance with the principles of the Declaration of Helsinki as revised in 2000. Subjects gave their written consent to the study.

Blood collection and analyses

Overnight fast blood was drawn from the men (12 hrs without food) in the morning at study entry and at the end of treatment. Moreover, men refrained from participating in any form of physical activity for 48 hrs before the study. Blood collection and handling were carried out under strictly standardized conditions and in line with manufacturer recommendations. Blood for clinical chemistry parameters and d-ROMs measurement was collected into evacuated tubes without anticoagulant, and then centrifuged for 15 mins at 1500x g. EDTA-plasma was used for lipoprotein isolation and paraoxonase activity determination.

Lipoprotein isolation, characterization and peroxidation

Lipoproteins were isolated from plasma as previously described [17]. After separation, lipoprotein were dialyzed and their levels of proteins, cholesterol (total and free), phospholipid and triacylglycerols, α-tocopherol and β-carotene were determined as previously described [16,22].

We incorporated cholesteryl (pyren-1-yl) hexanoate (PyrCE) or β-(pyren-1-yl) decanoyl γ-palmitoyl L-α phosphatidylcholine (PyrPC) in their monomeric forms into lipoprotein to measure the oxidizability of the hydrophobic core and surrounding amphipathic envelope, as previously described [16]. Labelled lipoproteins were resuspended (100 μg protein/mL) in phosphate-buffered saline pH 7.4, supplemented with 50 mM DTPA to chelate redox-active metal ions, and peroxidized at 37°C by incubation with AAPH 1 mM at 37°C [17]. The length of the lag phase (lag-time) and the velocity of the reaction in the propagation phase (slope) of peroxidation kinetic were calculated and used as indices of lipoprotein oxidizability [17].

Paraoxonase activities, total peroxyl radical-trapping antioxidant potential (TRAP) and reactive oxygen metabolites (d-ROMs)

Plasma PON-1 activities was assayed in plasma using two synthetic substrates: paraoxon (diethyl-p-nitrophenyl phosphate) and phenyl acetate. The activity toward paraoxon (paraoxonase activity) was calculated using a molar extinction coefficient of 18,290 M^{-1} cm^{-1}, whereas that toward phenyl acetate (arylesterase activity) was calculated using a molar extinction coefficient of 1310 M^{-1} cm^{-1}. The results are expressed in UmL^{-1}.

Plasma diluted to 5% in phosphate-buffered saline solution pH 7.4 (PBS) was used for the determination of total peroxyl radical–trapping potential (TRAP) as previously described using Trolox as reference standard [23].

The reactive oxygen metabolites (d-ROMs) assay was carried out by adapting the manufacturer's protocol to a 96-well-plate format. d-ROMs concentration of the samples was calculated using a serum standard and expressed as equivalent of H_2O_2 according to manufacturer's instruction.

Statistics

Since the Kolmogorov–Smirnov normality test revealed non-normal distribution of the parameters, results were presented as median SEM. The effects of hypo-caloric diet were analyzed by paired comparison (values before *vs.* after the intervention) using Wilcoxon tests. Two-tailed p-values ≤ 0.05 were considered significant. The linear relationships between the covariates were assessed using non-parametric Spearman Rank Correlation. A p-value ≤ 0.05 was considered statistically significant. All statistical analyses were performed by using StatistiXL software (version 1.5; StatistiXL, Western Australia).

Results

The hypocaloric diet determined a weight reduction of 7.4 0.6% in initial weight and significantly decreased BMI by 2.6 0.2 Kg/m^2. In Table 1 are summarized the anthropometric, blood pressure and blood parameter values before and after weight loss. All the risk factors associated to MetS improved significantly, with the exception of HDLs cholesterol, which was unchanged. In addition, the diet treatment improved total cholesterol, insulin, HOMA-IR, and CPR.

	T0	T1
BMI (kg/m^2)	33.7 ± 0.8	30.6 ± 0.7**
Waist circumference (cm)	110 ± 2.5	104 ± 1.8**
Systolic blood pressure (mmHg)	140 ± 3.9	126 ± 3.5*
Diastolic blood pressure (mmHg)	90 ± 2.4	80 ± 2.4**
Glycaemia (mg/dL)	101 ± 5.2	93 ± 4.3*
Insulin (μU/L)	11.4 ± 1.5	6.8 ± 1.0*
HOMA-IR	2.6 ± 0.4	1.7 ± 0.3**
Triacylglycerols (mg/dL)	122 ± 18	104 ± 10.0*
HDL cholesterol (mg/dL)	44 ± 2.8	47 ± 2.6
Cholesterol (mg/dL)	196 ± 6.8	170 ± 7.5*
Uric acid (mg/dL)	6.7 ± 0.4	6.4 ± 0.4
CRP (mg/L)	0.4 ± 0.1	0.1 ± 0.1*
Note: Data are expressed as median ± SEM; *p ≤ 0.05; **p ≤ 0.01.		

Table 1: Anthropometric characteristics, blood pressure and blood parameters of subjects before (T0) and after (T1) weight loss.

As expected, a hypocaloric diet changed dramatically also lipoprotein chemical composition (Table 2). Weight loss significantly decreased the protein concentration of LDLs, while it increased that of HDLs. As regards LDLs, because almost all of its protein content consists of one molecule of apo B-100, the decrease in protein levels is indicative of a reduction in the concentration of these lipoproteins. On the other hand, the changes of protein concentrations in HDLs can be ascribed to both variations of lipoprotein levels and modifications of Apo contents. Weight loss changed also the lipid composition, in particular the concentration of TAG, which significantly increased in LDLs while decreasing in HDLs. To understand better the variation in the composition of lipoproteins, we calculated the relative percentage of lipid weight distribution to total lipid mass. The changes of TAG levels significantly influenced the relative distribution of the other lipids. In particular, we observed a significant decrease in the percentage of phospholipids and both free and esterified cholesterol in LDLs, and a significant increase in the percentage of phospholipids and cholesteryl esters in the in HDLs. The proportions between total neutral lipids and beta-carotene as well as the ratio between alpha-tocopherol and phospholipids were not significantly influenced by weight loss (data not shown).

	LDL		HDL	
	T0	**T1**	**T0**	**T1**
Protein (mg/dL)	123 ± 10	105 ± 8*	82 ± 20	100 ± 29*
FC (mg/dL)	19.4 ± 1.4	18.8 ± 1.4	5.1 ± 0.4	5.0 ± 0.4
CE (mg/dL)	83.4 ± 6.1	81.0 ± 5.9	39.3 ± 2.9	39.8 ± 2.9
TAG (mg/dL)	44.6 ± 3.1	57.0 ± 4.6**	31.1 ± 2.3	25.3 ± 1.8**
PL (mg/dL)	77.2 ± 4.6	68.2 ± 4.9	49.7 ± 3.7	47.4 ± 3.3
CE/TAG	1.79 ± 0.1	1.43 ± 0.1**	1.36 ± 0.1	1.75 ± 0.1**
PL/FC	3.83 ± 0.1	3.69 ± 0.1	9.68 ± 0.1	9.20 ± 0.1*
FC (%w/w)	8.7 ± 0.1	8.3 ± 0.1**	4.2 ± 0.1	4.5 ± 0.1**
CE (%w/w)	37.3 ± 0.5	35.7 ± 0.6**	31.8 ± 0.5	34.4 ± 0.4**
TAG (%w/w)	20.9 ± 0.5	25.5 ± 0.6**	23.8 ± 0.4	19.6 ± 0.4**
PL (%w/w)	33.3 ± 0.3	31.6 ± 0.4**	40.0 ± 0.4	41.5 ± 0.4*

Note: Data are expressed as median ± SEM; * p ≤ 0.05; **; p ≤ 0.01; TAG: Triacylglycerols; PL: Phospholipids; FC: Free Cholesterol; CE Cholesteryl Esters.

Table 2: Protein (Apo) and lipid concentrations of lipoproteins before (T0) and after (T1) weight loss.

The values of parameters indicative of the oxidative status of LDLs and HDLs are reported in Figure 1. Panel A of Figure 1 depicts the resistance to oxidation (lag-time of the curves of peroxidation kinetic) of hydrophobic core and amphiphilic surface of both LDLs and HDLs before and after diet-induced weight loss. At baseline, the values of lag-time were similar in the core and surface of LDLs as well as in the core and surface of HDLs; however, the resistance to oxidation of LDLs is significantly higher than that of HDLs. After weight loss, resistance to oxidation of the core changed significantly and in the opposite direction: the value of lag-time of core increased in LDLs while it decreased in HDLs. However, in the surface there was only a significant reduction of lag-time of LDLs, while that of HDLs remained almost unchanged. Panel B reports the peroxidation rate (slope of the curve of peroxidation kinetic) of hydrophobic core and amphiphilic surface of both LDLs and HDLs before and after diet-induced weight loss. Weight loss influenced only the peroxidation rate of HDLs core by significantly decreasing and thus improving the slope of the peroxidation curve.

The susceptibility to oxidation of lipoproteins significantly correlated with the chemical compositions of LDLs and HDLs. In the hydrophobic cores, the duration of lag-time correlated directly with CE/TAG ratio (R=0.68, p=0.0006 and R=0.51, p=0.0174; respectively) and indirectly with the percentage of TAG (R=-0.66, p=0.0011 and R=-0.53, p=0.0125 respectively) and the TAG/PL ratio (R=-0.45, p=0.0411 and R=-0.44, p=0.0446; respectively). Moreover, the slope of LDLs correlated negatively with the protein levels (R=-0.46, p=0.0342), as well as the lag-time of HDLs with the concentration of PL (R=-0.48, p=0.0293). In the surface, the lag-time of HDLs correlated negatively with the concentration of protein and PL (R=-0.48, p=0.0289 and R=-0.49, p=0.0250 respectively).

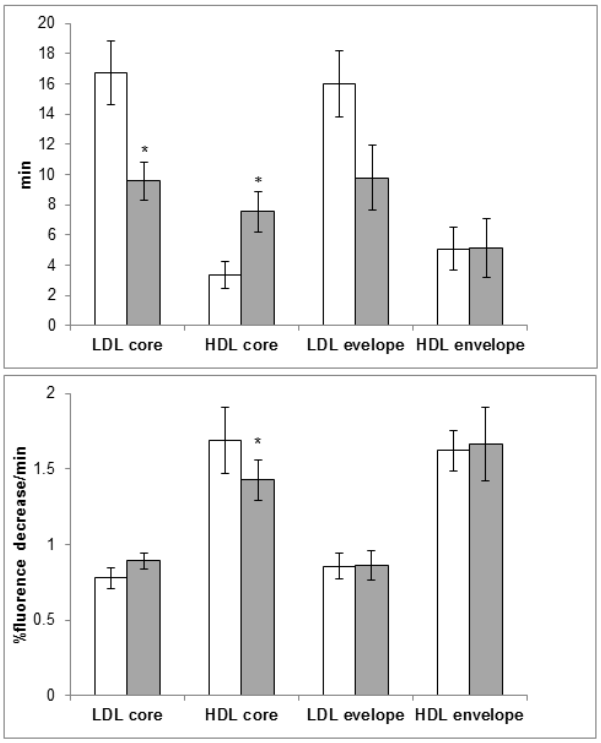

Figure 1: Values of lag-time (panel A) and slope (panel B) of the peroxidation kinetic curves in the hydrophobic core and amphiphilic surface of LDLs and HDLs before (blank bars) and after (grey bars) diet-induced weight loss.

Paraoxonase activities, reactive oxygen metabolites (d-ROMs) levels and peroxyl radical–trapping potential (TRAP) of plasma are reported in Table 3. Weight loss has led to a slight, but not significant, increase in both PON-1 activities (paraoxonase and arylesterase). d-ROMs and TRAP seem not to have been affected by weight loss. However, because uric acid is one of the major contributors to TRAP and weight loss determined a slight, though not significant, decrease in its concentration (Table 1), we calculated the impact of uric acid on TRAP, assuming that 1 mole of uric acid traps 1.34 moles of AAPH radicals [24]. The TRAP values without uric acid contribution (TRAP-U) were increased after weight loss but they did not reach statistical significance.

	T0	T1
Paraoxonase (U/mL)	117 ± 23.9	108 ± 19.5
Arylesterase (U/mL)	69.7 ± 4.6	69.6 ± 3.3
ROS (mg/dL H_2O_2)	27.6 ± 0.8	28.1 ± 0.5
TRAP (TE)	2222 ± 224.6	2033 ± 119.9
TRAP- U (TE)*	1674.9 ± 224.3	1745.8 ± 102.8
Note: Data are expressed as median ± SEM; * TRAP values without uric acid contribution, calculated by subtracting the radical trapped by uric acid assuming that 1 mole of uric acid trap 1.34 moles of AAPH radicals.		

Table 3: PON-1 activities, reactive oxygen species (ROS) and total peroxyl radical–trapping potential (TRAP) of plasma before (T0) and after (T1) weight loss.

Discussion

In the present study, we found that changes in lipoprotein chemical composition obtained through diet-induced weight loss had opposite effects on the oxidizability of LDLs and HDLs in overweight and I grade obese men with MetS, in particular at the level of their hydrophobic cores.

Weight loss significantly changed the concentrations of proteins and lipids in lipoproteins. In particular, we have observed that the protein concentration decreased in LDLs, while increased in HDLs. These changes are in alignment with previous studies showing that weight reduction decreases the concentrations of Apo B-100 [25] and increases the levels of Apo A-I [26] in subjects with MetS. As regards lipids, apart from a decrease in the levels of total TAG in plasma, we also observed a massive transfer of TAG from HDLs toward LDLs and, consequently, changes in the relative distribution of the other lipids. In HDLs, the decrease of TAG percentage was accompanied by a significant increase of percentage of both CE in the hydrophobic core and surface lipids. On the other hand, in LDLs the increase of TAG percentage was associated with a significant decrease of percentage of both CE and surface lipids. As suggested by previous studies showing that lipoprotein dimensions are inversely related to their TAG content [11,27], these changes are compatible with an increase of HDL dimensions and a reduction of LDL size. All these variations are the result of changes in the activity and/or concentration of proteins and enzymes involved in lipoprotein metabolism. Previous studies have demonstrated that weight loss induces in subjects with MetS: a) a decrease in concentration of apo C-III promoting the action of lipoprotein lipase and the consequent decrease of triglyceridemia [25]; b) a reduction of catabolism of apo A-I, an activator of lecithin cholesterol acyl transferase (LCAT, an enzyme that enrich the HDL core of cholesteryl ester) [26]; and c) a decrease in the mass of both cholesteryl ester transfer protein (the major lipid transfer protein in serum that collects triglycerides from VLDLs or LDLs and exchanges them for CE from HDLs, and vice versa) and phospholipid transfer protein [28,29].

As regards lipoprotein oxidizability, at baseline, the duration of lag-time (indicative of the resistance to peroxidation) of the surface was similar to this of the core in LDLs as well as in HDLs, but LDLs had a higher resistance to peroxidation than HDLs. Similarly, the slopes (indicative of the propagation rate of the peroxidation processes) of the surface was similar to that of the core in LDLs as well as in HDLs, but the propagation rates of peroxidation in LDLs were lower than in HDLs. Collectively, these results indicate that the lipid radicals derived from AAPH-induced reactions had the same chance of propagating peroxidation towards the adjacent lipids of both the cores and the surfaces and, moreover, that HDLs were more susceptible to oxidation than LDLs in our cohort study.

The changes of lipid composition influenced significantly the oxidizability of LDLs and HDLs but in opposite ways: the first got worse, while the latter were improved. In fact, in the LDL core, the increased ratio between TAG and CE approximately halved the resistance to peroxidation, while in the HDL core the reduction of this ratio doubled the value of lag-time. The direct correlation that we found between CE to TAG ratio and the duration of lag-time in the hydrophobic cores of LDLs and HDLs is further confirmation of the key role played by TAG in the processes of peroxidation of lipoproteins. In addition, hypocaloric diet did not influence proportions between total neutral lipids and beta-carotene and between alpha-tocopherol and phospholipids. It is well known that the peroxidation kinetic of lipoproteins is not only influenced by the concentration of pro-oxidants, oxidizable lipids and antioxidants, but also by the complex interactions that are established between these factors. At lipid level, the fluidity of the microenvironment in which a chemical reaction occurs strongly influences the interactions between the molecules involved in the process: the greater the degree of fluidity, the greater the likelihood of interaction between molecules. TAG are more oxidizable than CE [30] and has been shown to be directly correlated to the degree of fluidity of lipoprotein hydrophobic cores [17] because of their conformation and a higher content in unsaturated fatty acids. The fluidizing and oxidizing actions of TAG in LDL core could be responsible also for the reduction of lag-time in LDL envelope after weight loss. Moreover, as mentioned above, lipoprotein

dimensions are inversely related to their TAG content and the size of LDLs and HDLs seems to be related to their oxidizability: the higher the dimension, the lower the oxidizability [11,27].

Differently than in LDLs and HDLs, the plasma oxidative stress status - assessed by measuring TRAP (an index of the cooperative activity of different plasma antioxidant) and d-ROMS (indicative of oxidative damage to serum lipid) has not been significantly influenced by weight loss. Moreover, these results seem to indicate that the reduced oxidizability of HDLs is counterbalanced by the increased oxidizability of LDLs at plasma level. Our findings agree with Crujeiras et al who have not found significant variations of plasma total antioxidant capacity and concentration of malondialdehyde (a marker of lipid peroxidation) in obese women (BMI: 34.9 2.9 kg/m^2) treated with a hypocaloric diet that lead to a weight loss similar to that obtained by our subjects [31]. Nevertheless, several other studies in which different therapeutic approaches were used and diverse oxidative stress markers were measured have shown a positive effect of weight loss on oxidative stress status in subjects with different grade of obesity [31-33]. However, different modes of therapeutic intervention to achieve weight loss may lead to differing effects on oxidative stress status. In addition, the arylesterase and paraoxonase activities of PON-1 did not change after weight loss. However, the activity of this HDL-associated enzyme has been shown to be mainly influenced by genetic polymorphisms [7].

Conclusion

Although this study provides useful new information on the oxidizability of the hydrophobic core and amphipathic surface of lipoproteins after weight loss and highlights the important, and often underestimated, role of TAG in lipoprotein oxidizability, some limitations may also be discussed. First, the study population was relatively small and it was limited to male subjects. Therefore, the conclusions should not be generalized to all patients with MetS. Lastly, although it is recognized that the oxidizability of lipoproteins may play a role in the development of atherosclerosis, the validity of assay performed *in vitro* to predict the *in vivo* progression of atherosclerosis has yet to be proven.

In conclusion, more anti-atherogenic HDLs, but also more oxidizable LDLs seem to accompany the improvement of different metabolic factors induced by weight loss in obese men with MetS. This different response to weight loss is mainly related to the content of TAG in lipoprotein hydrophobic core. Thus, besides the well-established role of hypertriglyceridemia and its associated condition as risk factor for CVD, our results seem to indicate a potential direct involvement of lipoprotein TAG in the development of atherosclerosis. In addition, collectively our findings suggest that detailed knowledge of the oxidizability of both the surface and core of lipoproteins could be an additional means to clarify the mechanisms that links the changes of lipoprotein metabolism at risk of CVD in patients with MetS. Moreover, these parameters could be useful biomarkers in future studies on new dietary and therapeutic approaches for the treatment of obese subjects with MetS.

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References

1. Mackness B, Mackness M (2012) The antioxidant properties of high-density lipoproteins in atherosclerosis. Panminerva Med 54: 83-90.
2. Kontush A, Chapman MJ (2010) Antiatherogenic function of HDL particle subpopulations: Focus on antioxidative activities. Curr Opin Lipidol 21: 312-318.
3. Bowry VW, Stanleyt KK, Stocker R (1992) High density lipoprotein is the major carrier of lipid hydroperoxides in human blood plasma from fasting donors. Proc Nati Acad Sci 89: 10316-10320.
4. Shao B, Heinecke JW (2009) HDL lipid peroxidation, and atherosclerosis. J Lipid Res 50: 599-601.
5. Van der Stoep M, Korporaal SJ, Van Eck M (2014) High-density lipoprotein as a modulator of platelet and coagulation responses. Cardiovasc Res 103: 362-371.
6. Riwanto M, Landmesser U (2013) High density lipoproteins and endotelial functions: Mechanistic insights and alterations in cardiovascular disease. J Lipid Res 54: 3227-3243.
7. Mackness M, Mackness Avenida Príncipe BD, Platja M (2015) Human paraoxonase-1 (pon1): Gene structure and expression, promiscuous activities and multiple physiological roles. Gene 567: 12-21.
8. Ferretti G, Bacchetti T (2012) Effect of dietary lipids on paraoxonase-1 activity and gene expression. Nutr Metab Cardiovasc Dis 22: 88-94.
9. Feng H, Li XA (2009) Dysfunctional high-density lipoprotein. Curr Opin Endocrinol Diabetes Obes 16: 156-162.
10. Francis GA (2000) High density lipoprotein oxidation: In vitro susceptibility and potential in vivo consequences. Biochim Biophys Acta 1483: 217-235.
11. Ståhlman M, Fagerberg B, Adiels M, Ekroos K, Chapman JM, et al. (2013) Dyslipidemia, but not hyperglycemia and insulin resistance, is associated with marked alterations in the HDL lipidome in type 2 diabetic subjects in the DIWA cohort: Impact on small HDL particles. Biochim Biophys Acta-Mol Cell Biol Lipids 1831: 1609-1617.
12. Scheffer PG, Bakker SJL, Popp-Snijders C, Heine RJ, Schutgens RBH, et al. (2001) Composition of LDL as determinant of its susceptibility to in vitro oxidation in patients with well-controlled type 2 diabetes. Diabetes Metab Res Rev 17: 459-466.
13. Cicero AFG, Nascetti S, López-Sabater MC, Elosua R, Salonen JT, et al. (2008) Changes in LDL fatty acid composition as a response to olive oil treatment are inversely related to lipid oxidative damage: The EUROLIVE study. J Am Coll Nutr 27: 314-320.
14. Frei B, Gaziano JM (1993) Content of antioxidants, preformed lipid hydroperoxides, and cholesterol as predictors of the susceptibility of human LDL to metal ion-dependent and independent oxidation. J Lipid Res 34: 2135-2145.
15. Talayero BG, Sacks FM (2011) The role of triglycerides in atherosclerosis. Curr Cardiol Rep 13: 544-552.
16. Viani P, Cazzola R, Cervato G, Gatti P, Cestaro B (1996) Pyrene lipids as markers of peroxidative processes in different regions of low and high density lipoproteins. Biochim Biophys Acta 1315: 78-86.
17. Cazzola R, Cassani E, Barichella M, Cestaro B (2013) Impaired fluidity and oxidizability of HDL hydrophobic core and amphipathic surface in dyslipidemic men. Metabolism 62: 986-991.
18. O'Neill S, O'Driscoll L (2015) Metabolic syndrome: A closer look at the growing epidemic and its associated pathologies. Obes Rev 16: 1-12.
19. Rashid S, Watanabe T, Sakaue T, Lewis GF (2003) Mechanisms of HDL lowering in insulin resistant, hypertriglyceridemic states: The combined effect of HDL triglyceride enrichment and elevated hepatic lipase activity. Clin Biochem 36: 421-429.
20. Wing RR, Lang W, Wadden TA, Safford M, Knowler WC, et al. (2011) Benefits of modest weight loss in improving cardiovascular risk factors in overweight and obese individuals with type 2 diabetes. Diabetes Care 34: 1481-1486.

21. Alberti KGM, Zimmet P, Shaw J, IDF Epidemiology Task Force Consensus Group (2005) The metabolic syndrome-a new worldwide definition. Lancet 366: 1059-1062.
22. Cazzola R, Cervato G, Cestaro B (1999) Variability in alpha-tocopherol antioxidant activity in the core and surface layers of low- and high-density lipoproteins. J Nutr Sci Vitaminol 45: 39-48.
23. Cazzola R, Cestaro B (2011) Red wine polyphenols protect n−3 more than n−6 polyunsaturated fatty acid from lipid peroxidation. Food Res Int 44: 3065-3071.
24. Valkonen M, Kuusi T (1997) Spectrophotometric assay for total peroxyl radical-trapping antioxidant potential in human serum. J Lipid Res 38: 823-833.
25. Chan DC, Watts GF, Ng TWK, Yamashita S, Barrett PHR (2008) Effect of weight loss on markers of triglyceride-rich lipoprotein metabolism in the metabolic syndrome. Eur J Clin Invest 38: 743-751.
26. Richard C, Coulture P, Desroches S, Lichtenstein AH, Lamarche B (2013) Effects of weight loss, indipendent of change in diet composition, on apolipoprotein Al kinetic in men with metabolic syndrome. J Lipid Res 54: 232-237.
27. Suzukawa M, Abbey M, Howe PR, Nestel PJ (1995) Effects of fish oil fatty acids on low density lipoprotein size, oxidizability, and uptake by macrophages. J Lipid Res 36: 473-484.
28. Laimer MW, Engl J, Tschoner A, Kaser S, Ritsch A, et al. (2009) Effects of weight loss on lipid transfer proteins in morbidly obese women. Lipids 44: 1125-1130.
29. Tzotzas T, Dumont L, Triantos A, Karamouzis M, Constantinidis T, et al. (2006) Early decreases in plasma lipid transfer proteins during weight reduction. Obes 14: 1038-1045.
30. Rumley AG, Woodward M, Rumley A, Rumley J, Lowe GDO (2004) Plasma lipid peroxides: relationships to cardiovascular risk factors and prevalent cardiovascular disease. QJM 97: 809-816.
31. Crujeiras AB, Parra MD, Rodríguez MC, Martínez de Morentin BE, Martínez JA (2006) A role for fruit content in energy-restricted diets in improving antioxidant status in obese women during weight loss. Nutrition 22: 593-599.
32. De La Iglesia R, Mansego ML, Sánchez-Muniz FJ, Zulet MA, Martinez JA (2014) Arylesterase activity is associated with antioxidant intake and paraoxonase-1 (pon1) gene methylation in metabolic syndrome patients following an energy restricted diet. EXCLI J 13:416-26.
33. De La Iglesia R, Lopez-Legarrea P, Celada P, Sánchez-Muniz FJ, Martinez JA, et al. (2013) Beneficial effects of the RESMENA dietary pattern on oxidative stress in patients suffering from metabolic syndrome with hyperglycemia are associated to dietary TAC and fruit consumption. Int J Mol Sci 14: 6903-6919.