Myristic acid is associated to low plasma HDL cholesterol levels in a Mediterranean population and increases HDL catabolism by enhancing HDL particles trapping to cell surface proteoglycans in a liver hepatoma cell model

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1. Introduction

Dietary supplementation of unsaturated fatty acids (FA) protects against atherosclerosis and cardiovascular (CV) events [1]. The effects of dietary FA on the lipid metabolism have been widely investigated: mono- and poly-unsaturated FA (MUFA/PUFA) supplemented to the diet were effective in lowering total cholesterol (TC) and LDL cholesterol (LDL-C), while saturated FA (SFA) increased TC and LDL-C [2,3]. HDL cholesterol (HDL-C) showed a slight decrease with PUFA dietary supplementation [3] or a slight increase with any FA in other studies [2]. Plasma levels of PUFA, but not SFA, were associated with lower apolipoprotein Al (apo-AI) and HDL-C levels in the Framingham study, but with different features in men and women [4]. Animal models showed inconsistent results. PUFA and MUFA were both effective in decreasing the TC and LDL-C levels compared to SFA in some models, but HDL-C levels were decreased mainly by PUFA [5,6].

The control of HDL-C plasma levels involves different pathways...
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expression [8] while rats fed with PUFA increased it [14]. PPAR gamma-RXR heterodimer [13], nevertheless studies evalu-
elsewhere [15,16].

LDL-C was calculated by the Friedewald formula. Detailed pro-
cedures (Roche Diagnostics, Basel Switzerland) on a COBAS
MIRA plus auto-analyzer (Roche Diagnostics, Basel Switzerland).

ABCA1 via speci
pholipids transfer protein (PLTP), iii) clearance from the circulation
lecithin cholesterol transfer protein (LCAT) and/or lipid transport
lipoprotein lipase (LPL), hepatic lipase (HL) endothelial lipase (EL),
member 1, ABCA1 and scavenger receptor class I type B1, SRB1), ii)
modulate LPL [9], CETP [10] and HL [11]. The control of the lipid
metabolism by NEFA is mediated by the modulation of the Perox-
osome Proliferator—Activated Receptors (PPARs) activities [12]. The
hepatic HDL receptor, SR-B1, appears to be under the control of
PPAR gamma-RXR heterodimer [13], nevertheless studies evalu-
ating the regulation of SR-B1 expression gave contrasting results.
Unsaturated FA in macrophages and liver cells did not alter SR-B1
expression [8] while rats fed with PUFA increased it [14].

The first aim of this paper was to evaluate the relationship be-
tween plasma NEFA composition and HDL-C levels in a population
of Southern Italy from the “Ventimiglia Hearth Study”, an epide-
miological project started in 1989 with the aims to survey a typical
rural Mediterranean population in search of CV risk factors and to
register CV events over the years of follow up [15,16]. In the second
part of the study the NEFA significantly correlated with plasma
HDL-C levels in this population were investigated “in vitro” for their
ability to modify HDL catabolism using cultured liver hepatoma
cells (HepG2). HDL binding and/or internalization into liver cells
were investigated after conditioning them with different synthetic
FAs. Data concerning the effect of different FAs on SR-B1 expression
and the heparan sulphate proteoglycans (HSPG) contribution to
“in vitro” HDL catabolism are also presented.

2. Methods

2.1. Study subjects

The subjects of the present study were selected from the data-
base of the Epidemiological project “Ventimiglia di Sicilia Heart
Study” [15,16]. About nine hundreds sera were stored in 2002–2003 for epidemiologic analyses and were analyzed within a
year from storage. In the present study, a subset of the sampled
population was selected using a block randomization procedure
in order to represent the original population in terms of age, gender,
BMI and type 2 diabetes mellitus distribution. Randomization was
performed with a 1:5 ratio, so that about two hundreds subjects
were selected for subsequent analyses.

The adopted procedures were in accordance with the Helsinki
Declaration of 1975, as revised in 1983 and were approved by the
Ethical Committee of the University of Palermo. All the subjects
gave their informed consent to participate to the study.

2.2. Laboratory analyses

Lipid profile, blood glucose and other relevant biochemical pa-
rameters were measured using standard enzymatic-colorimetric
procedures (Roche Diagnostics, Basel Switzerland) on a COBAS
MIRA plus auto-analyzer (Roche Diagnostics, Basel Switzerland).
LDL-C was calculated by the Friedewald formula. Detailed pro-
cedures of the epidemiological study methodologies are described
elsewhere [15,16].

NEFA were measured in the plasma by Gas Chromatography-
Mass Spectrometry (GC/MS). NEFA were extracted from the
plasma adapting the method described by Lepage and Roy [17].
Plasma aliquots used for NEFA determination were stored after
addition of a mixture of an anti-proteases cocktail (Roche Diag-
nostic, Basel Switzerland) and 50 μM beta-hydroxy-toluene and
analyzed within a year from storage. Random samples were occa-
sonally retested to check for consistency of NEFA determination
after prolonged storage. Twenty five μL of plasma were used, since
the recovery of NEFA was linear up to 50 μL of plasma (data not
shown). The extraction procedure was checked for possible release
of fatty acids from triglycerides by spiking 5 μL of Glycerol
Tri(hexadecanoate-1-13C), (Sigma Aldrich, MO, U.S.A.) in some
samples. The amount of 1–13C hexadecanoate (C16:0, palmitate)
released during the extraction procedure was negligible (data not
shown). Extracted NEFA were converted to methyl ester (FAME)
and injected in a Hewlett Packard (Palo Alto, CA, U.S.A.) 5960 Gas
chromatograph equipped with a 100 m × 0.25 mm SP2560 column
(Supelco, Bellefonte, U.S.A.) and coupled to a Hewlett Packard 5973
inert Mass spectrometer. GC/MS conditions were: injection split to
a 1:30 ratio, injector temperature 180 °C. The oven initial temper-
ature was 80 °C for 4 min, and then it increased to 220 °C at 8 °C
min and remained at 220 °C for 32 min. All reagents and standards
were purchased from Sigma–Aldrich (Sigma–Aldrich, MO, U.S.A.).

MS was used in Single ion monitoring (SIM mode). Eleven m/z
characteristic of saturated, mono-di- tri-tetra-enoic or n-enoic fatty
acids were selected as described in the “AOCs lipid library” site
according to Christie W. et al. (http://lipidlibrary.aocs.org/ms/
massspec.html).

Calibration curves were obtained by using true fatty acids
standards (FAME, Supelco, Sigma–Aldrich), heptadecanoic acid
(C17:0) was used as internal standard (Sigma–Aldrich). All cali-
bration curves were highly linear between 0 and 20 μg of injected
FAME, (all Pearson’s R’s were >0.996, data not shown); all cali-
bration points were prepared in triplicate spiking normal plasma
with scalar amount of standards and the curves was accepted if the
coefficients of variation for the repeated measures were below 5% (data not shown).

2.3. Lipoproteins isolation and fluorescent labeling

Normal lipoproteins were isolated from a large pool obtained by
young healthy blood donors. Plasma was aliquoted and stored after
the addition of the anti-proteases and anti-oxidant cocktail
described before. Plasma HDL fractions were obtained by sequen-
tial ultracentrifugation in the 1.063–1.210 g/mL density range in a
L90 K Optima Beckman–Coulter Ultracentrifuge (Beckman Coulter,
Fullerton, CA, U.S.A.). HDL particles were labeled with DIL (di-
(2,7'-dichlorofluorescein)-(3',3',5',5'-tetraethylfluoresceine iodide) as described elsewhere [18].
In order to label HDL CE, recombinant HDLs containing BODIPY
FL12 (a fluorescent analog of cholesteryl esters (CE) from Molecular
Probes-Life Technologies, Eugene, OR (U.S.A.) were prepared as
described elsewhere [19]. The BODIPY FL12 molecule is actively
hydrolyzed within lysosomes, but not by cholesteryl esterase.

2.4. Cell cultures and FA conditioning

HepG2 hepatoma cells were grown at 37 °C, 5% CO2 in Dul-
becco’s modified Eagle’s medium (DMEM glutamam, Gibco BRL,
Gaithersburg, Maryland, United States) supplemented with 10% Fetal
Bovine Serum, 100 U/mL penicillin and 100 μg/mL strepto-
mycin (Gibco BRL). 1% non essential amino acids. Cells were grown
to sub-confluence and seeded in 6 or 24 wells plates (Corning Life
Sciences, MA, U.S.A.) as specified. In conditioning experiments FA
were first complexed to Bovine Serum Albumin (BSA, Sigma–
Aldrich, St. Louis, MO, U.S.A.) mixing a solution of 100 mM FA in
0.1 M NaOH with a 10% FA-free BSA solution prepared in H2O, at 55 °C for 15 min. The complexed FA were supplemented in the medium to achieve 0.5 mM in 1% BSA, or 0.25 mM when indicated. Non-conditioned cells were supplemented with BSA alone. Five fatty acids were used in all experiments: myristic acid (C14:0), palmitoleic acid (C16:1), stearic acid (C18:0), oleic acid (C18:1n9), linoleic acid (C18:2n6), all from Sigma–Aldrich.

2.8. SR-B1 protein expression on HepG2 hepatoma cells membrane by immuno-cytofluorimetry

The HepG2 were grown in 6 well plates to sub-confluence and conditioned for 24 h with FA as described above. The assays were performed in triplicate. After conditioning, cells were washed twice with PBS plus 2 mM CaCl2, 1 mM MgCl2 and trypsinized in 0.05% trypsin, 0.02% EDTA (Trypsin-EDTA solution 1 x, Sigma–Aldrich). Trypsin was inactivated by adding cell culture medium and the cells, collected in tubes, were centrifuged at 1000 g for 5 min. After two washes with PBS, the cells were suspended in 1% BSA in PBS. The cellular suspensions were incubated overnight at 4 °C with a 1:50 dilution of rabbit polyclonal anti SR-B1 antibody (donated kindly by Prof. Schonfeld G., Washington University di St. Louis, MO - U.S.A.) in PBS and 1% BSA followed by a 1 h incubation with a 1:100 dilution of a Alexa-fluor 488 conjugated secondary anti-rabbit antibody (Life Technologies). After a final wash, Cells were analyzed by the FACS Calibur using the FI1 channel and analyzed by CellQuest Pro software (BD) as before. Median values of fluorescence intensity (subtracted of cells autofluorescence) were considered as measures of surface SR-B1. Data were expressed as proportions of BSA conditioned cells.

2.9. Statistics

Differences in clinical and biochemical parameters in the population study were evaluated by Student’s T-test. Univariate correlations were evaluated by Pearson’s R, while independent correlations were evaluated by multiple regression analysis (MRA) after adjustment for confounders (age, gender, BMI), using a step-forward option. A set of step-backward MRA were performed in parallel with similar results (data not shown). Statistics were calculated by the SYSTAT 12 software (SYSTAT corp., CA, U.S.A.).

Kinetics curves of DIL-HDL binding to HepG2 cells were fitted with a Michaelis–Menten (MM) equation modified by adding a linear component in order to take into account a non-specific, non-saturable HDL binding pathway (Supplementary Equation (1)). Curve fitting was performed using the nls2 (“Nonlinear regression with brute force”) package from the R statistic software (https://cran.r-project.org/web/packages/nls2/index.html). The software calculated estimates of MM parameters with relative standard errors of estimated: Bmax expressed the amount of DIL-HDL bound to saturable sites on the cell surface, Km expressed the MM constant, and Kasp expressed the linear non-specific binding constant (see Supplementary Equation (1)). Differences in the estimated kinetics parameters between non-conditioned cells and FA conditioned cells were evaluated from calculated means and SD by Student’s T test.

3. Results

3.1. Relationship between HDL-C levels and plasma FA in a Mediterranean population

Table 1 shows clinical data and NEFA profile of the study population divided according to gender. The table shows that the only biochemical parameters significantly differing between males (n = 103) and females (n = 97) were TC, HDL-C and TG (nearly significant, p = 0.08), all of them higher in females. TG/HDL-C (mg to mg ratio) was higher in males. No differences in plasma NEFA proportions were detected with the exception of C18:2n6 (p = 0.03) and aggregated PUFA (p = 0.02) slightly higher in females.

Table 2 shows the correlations between HDL-C plasma levels and plasma NEFA relative amounts. At univariate analysis (column 2), HDL-C levels were correlated positively with PUFA (r = 0.28,


Biochemical parameters and plasma NEFA profiles in the Ventimiglia Heart Study population divided by gender.

* Student’s T-test p-values, Males vs. Females.

** Table 2 **
Correlation of plasma fatty acids composition with HDL cholesterol levels.

<table>
<thead>
<tr>
<th>Plasma NEFA</th>
<th>Pearson’s R (p value)*</th>
<th>Std coefficient (p value)**</th>
</tr>
</thead>
<tbody>
<tr>
<td>C14:0 (myristic)</td>
<td>−0.27 (&lt;0.01)</td>
<td>−0.24 (&lt;0.01)</td>
</tr>
<tr>
<td>C16:0 (palmitic)</td>
<td>−0.17 (0.06)</td>
<td>−0.19 (0.03)</td>
</tr>
<tr>
<td>C16:1 (palmitoleic)</td>
<td>−0.09 (0.18)</td>
<td>0.19 (0.03)</td>
</tr>
<tr>
<td>C18:0 (stearic)</td>
<td>−0.08 (0.27)</td>
<td>−0.22 (&lt;0.01)</td>
</tr>
<tr>
<td>C18:1n9 (oleic)</td>
<td>−0.27 (&lt;0.01)</td>
<td>Excl (0.96)</td>
</tr>
<tr>
<td>C18:2n6 (γ-linolenic)</td>
<td>0.01 (0.89)</td>
<td></td>
</tr>
<tr>
<td>C18:3n3 (α-linolenic)</td>
<td>−0.12 (0.12)</td>
<td></td>
</tr>
<tr>
<td>C20:1n9 (cis-11-Eicosenoic)</td>
<td>−0.24 (&lt;0.01)</td>
<td>−0.19 (0.01)</td>
</tr>
<tr>
<td>C20:2 (cis-11,14-Eicosadienoic)</td>
<td>−0.04 (0.58)</td>
<td></td>
</tr>
<tr>
<td>C20:3n6 (cis-8,11,14-Eicosatrienoic)</td>
<td>0.05 (0.47)</td>
<td></td>
</tr>
<tr>
<td>C20:4n6 (Arachidonic)</td>
<td>0.10 (0.16)</td>
<td></td>
</tr>
<tr>
<td>C20:5n3 (cis-5,8,11,14,17-Eicosapentaenoic)</td>
<td>0.03 (0.64)</td>
<td></td>
</tr>
<tr>
<td>C22:6n3 (cis-4,7,10,13,16,19-_docosahexaenoic)</td>
<td>−0.01 (0.87)</td>
<td></td>
</tr>
<tr>
<td>Aggregated NEFA</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SFA</td>
<td>−0.17 (0.01)</td>
<td>Excl (0.61)</td>
</tr>
<tr>
<td>MUFA</td>
<td>−0.27 (&lt;0.01)</td>
<td>Excl (0.68)</td>
</tr>
<tr>
<td>PUFA</td>
<td>0.42 (0.02)</td>
<td>Excl (0.96)</td>
</tr>
</tbody>
</table>

* Pearson’s R Univariate correlation.

** Multiple regression analysis adjusted for age and gender. Statistical p values in brackets.

p < 0.01) mainly because of C18:2n6 correlation (+0.28, p < 0.01) and negatively with MUFA (−0.27, p < 0.01) mainly because of C18:1n9 (−0.27, p < 0.01). SFA correlated negatively, but not significantly, with HDL-C levels because of the significant C14:0 correlation with HDL-C levels (−0.27, p < 0.01), while C16:0 and C18:0 did not correlate with HDL-C levels. NEFA that were correlated with HDL-C at univariate analysis were included in a multiple regression analysis (MRA) that also included the most abundant plasma NEFA, using age and gender as covariates. The MRA showed that only C14:0 (β = −0.24 p < 0.01), C18:1n9 (β = −0.22 p < 0.01) and C20:1n9 (cis-11-Eicosenoic, β = −0.19 p < 0.01) were independently and negatively correlated with HDL-C levels, while C16:1 was positively correlated (β = +0.19 p = 0.03) at MRA but not at univariate analysis. Correlation coefficients were not significantly affected by measures of adiposity, as B.M.I. and waist circumference (Supplementary Tables S–1).

3.2. Effects of FA conditioning on HDL catabolism in liver cultured cells (HepG2)

The FA used to condition HepG2 cells were selected among those found correlated with HDL-C levels in the population study or
resulted more abundant in the plasma with the exception of C20:1, because of the presence of C18:1 as similar MUFA. FA were tested “in vitro” in a liver hepatoma HepG2 cells model. HDL binding to cell surface was evaluated after conditioning the cell with single FA. Fig. 1 shows the kinetics of the DIL-HDL binding to cells surface after conditioning the cells with different FA. Individual FA data, fitted curves according to the first-order Michaelis–Menten model and estimated kinetic parameters are presented in Supplementary Fig. S1–S5. A summary of kinetics estimates is shown in Table 3. The data show that no FA significantly increased HDL binding by increasing the amount of HDL bound to saturable sites of cell membrane (Bmax), while C14:0 showed a peculiar behavior, increasing the HDL surface binding by increasing the amount of HDL bound to non-saturable sites (slope of linear fitting, kasp = 43%, p < 0.001) on cell surface. All other FA but C18:0 seemed to increase the affinity for cell surface binding sites (decrease of km constant).

3.3. HSPG contribution to HDL catabolism in liver cultured cells (HepG2) after conditioning with FA

In order to test the hypothesis that the HSPG moiety of hepatocytes surface might account for the peculiar kinetics of C14:0, DIL labeled HDL were competed with liver cells before and after treatment with heparinase I and III, an enzyme mixture able to hydrolyze cell surface HSPG. Fig. 2 shows that heparinases abolished about the 30% (p = 0.001) of total HDL binding in cells treated with C14:0, while no effect could be detected in other FA conditioned cells. All other FA reduced the loss of HDL binding after HSPG stripping shown in non-conditioned cells (~14%, p = 0.01), and C18:1n9 even increased (not significantly) HDL binding (per mg of cell protein) after HSPG cleavage (+9%, p = 0.18).

3.4. Effects of FA conditioning on HDL-CE internalization in liver cultured cells (HepG2)

Internalization of fluorescent CE was performed by incubating FA-conditioned cells with recombinant HDL containing BODIPY-FL12. Internalized CE were measured by flow cell fluorometry. FA did not modify the CE internalization with the exception of C14:0 that significantly increased CE internalization by 32% (p = 0.01) as shown in Table 3.

3.5. Effects of FA conditioning on SR-B1 cell-surface expression on liver cultured cells (HepG2)

The effect of FA on SR-B1 cell surface expression was evaluated by immuno-cytofluorimetry on FA conditioned HepG2 cells. No significant differences in SR-B1 expression were found. Still, SFA (C14:0, C18:0) showed a rough 20% reduction of SR-B1 expression, while C18:1n9 showed a non significant +12% increase (Table 3, p = 0.27).

4. Discussion

In this paper we first investigated how plasma NEFA composition correlated with plasma HDL-C levels in a free living population of Southern Italy. This population is a typical Mediterranean population following a “modern” version of the Mediterranean diet with a lower content of fibers and an excess of introduced calories in comparison with the “traditional” diet [15]. Olive oil constitutes the majority of dietary fat. This population is characterized by a high prevalence of obesity [15,16] and metabolic syndrome (MetS) [16] that also represents a relevant risk factor for CV events observed during a 15 years follow-up [16]. More, the average HDL-C levels of this population are slightly lower than the Italian levels [15,16].

We found that HDL-C levels of this population correlated positively with the proportion of plasma PUFA and negatively with SFA. Among individual NEFA, HDL-C levels strongly and negatively correlated with C14:0, C18:1n9 and C20:1n9, and positively with C16:1. Different studies showed that C14:0 positively correlated with plasma HDL-C levels, and a large meta-analysis including 60 dietary intervention studies concluded that the increase of HDL-C due to SFA progressively decreases with the elongation of the acyl chain, being maximal for C12:0 (lauric acid) and not relevant for C18:0 [2]. The dietary supplementation of C14:0 did not produce a significant raise in HDL-C levels in the same study [2]. Another large meta-analysis showed that the isocaloric replacement of C18:0 with C12:0/C14:0/C16:0 did not significantly raise the HDL-C levels [22]. These contrasting results might be explained by the heterogeneous characteristics of the studied populations. The population of the present study shows a high prevalence of MetS [16]. High levels of C14:0 were associated to the risk of MetS with an odds ratio of 5.84 in women and 2.23 in men in study of obese subjects [23]. It is possible that the inverse relationship between C14:0 and low HDL-C in obese subjects might be explained by the increase of MetS induced by C14:0, since low levels of HDL-C are characteristic of MetS patients [16].

Another explanation of these results might be related to the choice to assay NEFA instead of total FA in the present study. Dietary FA modify not only NEFA but also total FA composition, and the modification of esterified FA profile may result a better predictor of HDL levels than NEFA profile.

C18:1n9 was correlated with lower levels of HDL-C in our study, being oleic acid highly represented in the Mediterranean diet of this population [15], while C18:2n6 was associated with higher HDL-C levels, though the latter association was not maintained at
multivariate analysis.

We tried to understand if an altered hepatic catabolism of HDL induced by FA might contribute to explain the results found in the population study. We used an “in vitro” model of cultured hepatoma cells conditioned with those FA that resulted correlated (as NEFA) with plasma HDL-C levels in the population study (Table 2), or that resulted very abundant in the plasma, as C14:0. We decided to exclude C20:1n9 to avoid over-representation of MUFA, being C18:1 about ten-times more abundant than C20:1 and very similar to it in terms of chain length.

The main result of the “in vitro” part of the study consisted in the finding that myristic acid modified the HDL binding to liver cultured cells in a peculiar manner. C14:0 increased the non-specific binding of HDL to cell surface and it did not affect the saturable receptor-mediated binding (Fig. 1, Table 2 and Supplementary Figs. S–1). The increase of HDL binding caused by C14:0 cell conditioning showed a linear dose–response effect between 0.25 mM and 1 mM of supplemented C14:0 (data not shown). The increase of HDL binding is partly explained by the presence of HSPG, since their removal by heparinases treatment determined roughly a 30% loss of HDL binding activity in comparison with non-conditioned cells and all the other FA (Fig. 2). C14:0 conditioning also increased the internalization of fluorescent CE contained in synthetic HDL3 by +32% (Table 3). HSPG are an essential part of the lipoproteins-CE removal from the bloodstream. HDLs are trapped by the PG matrix surrounding the hepatocytes and stripped of their CE content by the HL in exchange of triglycerides (TG) [7]. This mechanism could explain why CE internalization in the hepatic cells was increased by C14:0, though this result is not consistent with previous reports. In a dietary intervention study, a diet rich in C14:0 and other SFA was associated with a reduced activity of HL, resulting in a positive correlation with LDL size [24]. Also, a diet supplementation with SFA in monkeys [5] determined an increase of plasma HDL-C and a decrease of CE hepatic content, suggesting that CE internalization was not increased but reduced in those models. The difference between the metabolism of the animal models and that of isolated cultured cells may explain this apparent discrepancy. Interestingly, high C14:0 levels were found associated with lower Lipoprotein[a] (Lp[a]) plasma levels [25]. Since C14:0 is probably not able to affect apo(a) synthesis, being apo(a) levels under strict genetic control [25], it is plausible that C14:0 reduces Lp(a) levels by increasing Lp(a) trapping by HSPG, a relevant mechanism involved in Lp(a) catabolism [26]. Our results suggests that C14:0 increases the catabolism of HDL-CE, Lp(a), and probably VLDL by modifying the proteoglycan extracellular matrix. We also evaluated the effect of FA on SR-B1 protein expression on the liver cells surface. We measured the surface-exposed SR-B1 by immuno-cytofluorimetry and we found that C14:0 and C18:0 showed a trend toward the decrease in SR-B1 expression (roughly by 20% though not significant, Table 3). If confirmed and validated, this result would be in accord with another study showing reduced a SR-B1 expression in hamsters treated with C14:0 [27]. Dietary interventions enriched in C14:0 increased circulating HDL-3 particles [28,29], that are generated by SR-B1 from nascent HDL [7], but the increase of HDL3 in these studies has been explained by the impairment of the HDL3→HDL2 conversion due to the lecithin-cholesterol acyl transferase (LCAT) inhibition induced by C14:0 [29].

All the results concerning the other investigated FA are less straightforward to interpret. C18:1n9 was associated with lower HDL-C levels in the population study confirming large association studies [2,3]. The mechanism might be related to an increase of HDL clearing related to SR-B1. We observed a not significant 12%
increase of SREB1 on the cell surface (Table 3), but this trend was supported by neither an increase in HDL particles bound to cell surface nor an increase in CE internalization under C18:1n9 cells conditioning. C18:1n9 is a powerful PPARs activator [12], so it is possible that the negative correlation with plasma HDL levels might be related to the effect of PPARs activation in liver cells, but it is not clear why C18:2n6, also a potent PPARs activator, did not show any relevant correlation with SR-B1 in this study.

The main limit of this study is that the “in-vitro” experiments were focused only on HDL particles catabolism by liver cells, and it is not always possible to find a straight correlation between the data obtained “in-vivo” from human studies and the limited catabolic aspects elucidated by the “in-vitro” experiments. In spite of this limitation, a novel mechanism by which C14:0 affects HDL-C catabolism is clearly demonstrated in cultured hepatoma cells.

In conclusion, we showed that myristic acid is negatively correlated with plasma levels of HDL-C in a Mediterranean population characterized by an excess of obesity and metabolic syndrome. We suggest that part of this correlation might be explained by an increase of HDL binding to hepatic HSPG and subsequent cholesteryl esters stripping by proteoglycans-bound lipases. Our study reinforces the necessity to reduce overweight and to decrease the amount of dietary saturated fats in order to improve population’s health.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.atherosclerosis.2015.12.036.

References