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Original article

Oxidative stress, HDL functionality and effects of intravenous iron administration in women with iron deficiency anemia

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SUMMARY

Background and aims: Iron deficiency anemia (IDA) affects around 20–30% of adults worldwide. An association between IDA and cardiovascular disease (CVD) has been reported. Oxidative stress, inflammation and low concentration of high-density lipoproteins (HDL) were implicated on endothelial dysfunction and CVD in IDA. We studied the effects of iron deficiency and of an intravenous iron administration on oxidative stress and HDL characteristics in IDA women.
Methods: Two studies in IDA women are presented: a case–control study, including 18 patients and 18 age-matched healthy women, and a follow-up study 72hr after the administration of intravenous iron (n = 16). Lipids, malondialdehyde, cholesteryl ester transfer protein (CETP), paraoxonase-1 (PON-1) and HDL chemical composition and functionality (cholesterol efflux and antioxidative activity) were measured. Cell cholesterol efflux from iron-deficient macrophages to a reference HDL was also evaluated.
Results: IDA patients showed higher triglycerides and CETP activity and lower HDL-C than controls (all $p < 0.001$). HDL particles from IDA patients showed higher triglyceride content (+30%,$p < 0.05$) and lower antioxidative capacity (−23%,$p < 0.05$). Although HDL-mediated cholesterol efflux was similar between the patients and controls, iron deficiency provoked a significant reduction in macrophage cholesterol efflux (−25%,$p < 0.05$). Arylesterase activity of PON-1 was significantly lower in IDA patients than controls (−16%,$p < 0.05$). The intravenous administration of iron was associated with a decrease in malondialdehyde levels and an increase in arylesterase activity of PON-1 (−22% and +18%, respectively, $p < 0.05$).
Conclusion: IDA is associated with oxidative stress and functionally deficient HDL particles. It remains to be determined if such alterations suffice to impair endothelial function in IDA.

1. Introduction

Iron deficiency is a silent condition that causes anemia only when hemoglobin (Hb) synthesis results impaired [1]. Iron deficiency represents one of the major causes of anemia and, specifically, iron deficiency anemia (IDA) affects around 20–30% of adults worldwide [2]. Inadequate iron intake, impaired absorption or transport, and physiologic or pathologic blood loss are generally associated with IDA [1,3].

Among the health impacts of anemia, an association between low Hb concentration and increased risk of cardiovascular disease

Abbreviations: IDA, iron deficiency anemia; Hb, hemoglobin; MDA, malondialdehyde; HDL, high-density lipoprotein; CETP, cholesteryl ester transfer protein; TG, triglyceride; LDL, low-density lipoprotein; hsCRP, high sensitivity C-reactive protein; apo, apolipoprotein; PON, paraoxonase activity; ARE, arylesterase activity; Lp-PLA_2, lipoprotein-associated phospholipase A_2.

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(CVD) has been observed in the ARIC, VA-HIT and AMORIS studies [4–6]. In addition, the DAN-MONICA [7] study showed a steady increase in CVD risk in healthy women with ferritin levels lower than a threshold level (~50 μg/l). It has been proposed that increased sympathetic nervous activity, overactivation of the renin-angiotensin axis, a proinflammatory state and increased oxidative stress can underlie such association [4–6]. Of critical importance, all these conditions can trigger endothelial dysfunction, one of the early events in atherogenesis.

Supporting the hypothesis of a proinflammatory state in iron deficiency, IDA subjects showed elevated levels of the lipoprotein-associated phospholipase A_2 (Lp-PLA_2), an inflammatory marker, in comparison with controls [8]. Moreover, low hepcidin concentration, characteristic of IDA, was associated with an increased secretion of metaloproteases and inflammatory reactivity of macrophages [9,10].

Regarding oxidative stress, previous studies have shown higher malondialdehyde (MDA), and other markers of oxidative stress, as well as lower activity of antioxidant enzymes in IDA women *vs.* controls [11,12]. Among these antioxidant enzymes, the activity of the high-density lipoprotein (HDL)-bound paraoxonase-1 was also reduced in women with IDA [8,11]. However, measurements of HDL antioxidative activity have not been reported in IDA women.

HDL particles are heterogenous and display different anti-atherogenic properties, such as antioxidative and antiinflamatory properties [13]. However, under several metabolic conditions in which HDL composition is altered, its antiatherogenic properties can be compromised [13]. It is known that patients with insulin resistance possess functionally deficient HDL particles as a consequence of alterations in their lipid and protein composition. Such changes are mainly associated with elevated cholesteryl ester transfer protein (CETP) activity and involve HDL enrichment in triglycerides (TG) and depletion in cholesteryl esters (CE) [14,15]. As high CETP activity has been observed in IDA women in comparison with controls [8], both HDL composition and functionality can be altered in IDA.

Our aim was to study the effects of iron deficiency and of an intravenous iron administration on oxidative stress, inflammatory markers and HDL characteristics in IDA women. Two studies are presented: a case–control study and a follow-up study, which evaluated changes 72 h after the administration of intravenous iron.

2. Materials and methods

2.1. Subjects

Adult women with IDA were recruited at the Hematology Service of the Italian Hospital of Buenos Aires, Argentina. IDA was defined as: Hb concentration <12.0 g/dL, mean corpuscular volume <80 fL, red blood cell distribution width >15%, transferrin saturation <15% and ferritin <15 μg/l. Subjects presenting any of the following conditions were excluded: a) diagnosis of diabetes or any other endocrine pathology, b) liver or renal diseases, c) inflammatory pathologies which could be associated with anemia (inflammatory bowel disease, reumathoid arthritis, and cancer), d) acute inflammatory processes and/or high sensitivity C-reactive protein (hsCRP) > 10 mg/l, e) smoking or heavy alcohol drinking (>40 g/day), and f) antioxidant, oral contraceptive or drug consumption that could alter lipoprotein metabolism. All patients and controls gave their inform consent to participate in the study and the protocol was approved by the Institutional Review Boards from the Italian Hospital of Buenos Aires and from School of Pharmacy and Biochemistry, University of Buenos Aires.

2.1.1. Sample size

The sample size was calculated based on a previous study carried out in IDA women and healthy subjects [8]. The outcome variables chosen to perform the sample size calculation for this study were HDL-C and arylesterase (ARE) activity of the enzyme paraoxonase-1. Having defined a 0.8 power, an effect size of 1.0 and a significance level of 0.05, the number of patients to be included in the present studies was at least 17.

2.1.2. Case-control study

Eighteen IDA women were selected and compared with a control group of 18 age-matched healthy women. Controls were women who attended the same hospital center as the patients for a general medical examination. All control women had to present markers of glucose, iron and lipid metabolism within the reference ranges. Controls who presented any of the following conditions were not included: a) weight loss of unknown cause during the last 6 months, b) vegetarian diet, c) irregular menstrual periods, d) positive serology for any disease, e) infectious processes during the last month, f) any chronic inflammatory condition, g) smoking or heavy alcohol drinking (>40 g/day), and f) antioxidant, oral contraceptive or drug consumption that could alter lipoprotein metabolism. Before their inclusion, all the patients and controls completed a food frequency questionnaire in order to control for their dietary intakes of red meat, fruits, legumes and vegetables. Subjects with extremely high or low intake of any food group were also excluded. Blood samples were drawn after a 12 h overnight fast. General biochemical determinations were performed within 48 h and aliquots of serum were frozen at −70 °C for the other determinations.

2.1.3. Follow-up study

Sixteen women with IDA and indication of intravenous iron treatment prescribed by the hematology specialist were included in this study. The main reason for this indication was intolerance to oral iron supplementation. Baseline blood samples were drawn after a 12 h overnight fast. In all cases, the baseline samples were drawn from two to four days before the iron infusion. The infusion consisted in 300 mg/2 h of iron sucrose (Venofer®, Vifor Pharma, Glattbrugg, Suiza). After 72 h, 12 h fasting blood samples were drawn. Samples were used within 48 h for general biochemical determinations and aliquots of serum were frozen at −70 °C for the other determinations.

2.2. Anthropometric and general biochemical determinations

Height and weight were measured with the subject wearing light clothes and without shoes. Body mass index (BMI) was calculated and BMI categories defined according to the World Health Organization (WHO) adult definition. Blood pressure was determined with a random-zero sphingomanometer after 5 min of rest and the average of two measurements was recorded. Hypertension was defined in accordance to international guidelines.

Complete blood count was determined in a Coulter® LH-750 hematology analyzer (Beckman Coulter, USA). Transferrin, apolipoprotein (apo) A-I and apo B concentrations were measured in an IMMAGE® (Beckman Coulter, USA) nephelometer. Ferritin concentration was assayed by an electrochemiluminiscence assay (VITROS® ECiQ, Ortho-Clinical Diagnostics, USA). Serum levels of glucose, iron, TG, and total cholesterol (TC) were measured by standardized methods (Roche Diagnostics, Mannheim, Germany) in a COBAS® C501 autoanalyser (Roche Diagnostics, Mannheim, Germany). Low density lipoprotein (LDL) and HDL-C concentrations were determined by selective precipitation methods (Roche Diagnostics, Mannheim, Germany). Very low density lipoprotein-

cholesterol (VLDL-C) was calculated. Insulin concentration was measured by radioimmunoassay (DPC, Los Angeles, CA, USA) and homeostasis model assessment (HOMA)-IR was calculated. Coefficients of variation (CV) of all the general biochemical determinations were within acceptable parameters. The laboratory in which these measurements were carried out is certified by the College of American Pathologists (CAP). HsCRP concentration was measured by a high sensitivity immunoassay (Roche Diagnostics, Mannheim, Germany), intra and inter-assay CV were 2.5% and 3.1%, respectively. MDA was measured by a spectrophotometric method using thiobarbituric acid, after the precipitation of serum proteins with trichloric acid 20% w/v [16]. For MDA measurement, samples from each study were processed in the same run. Within run CV was 3.7%.

2.3. Activities of lipoprotein-associated proteins and enzymes

CETP activity was determined according to a modified endogenous assay [8]. Results were expressed as a percentage of ^{3}H-cholesteryl esters transferred from HDL3 to apoB-containing lipoproteins. Measurements were all carried out within the same assay, CV was 4.9%. Paraoxonase-1 activities were assessed using paraoxon and phenylacetate as substrates in order to measure paraoxonase (PON) and ARE activities, respectively [8]. Measurements were carried out within the same assay, CV was 5.5% and 4.8%, respectively. Lp-PLA_2 was measured according to the method previously described [8]. Measurements were carried out within the same assay, intra-assay CV was 5.1%.

2.4. Lipoprotein isolation

HDL (d:1.063–1.210 g/ml) was isolated from serum by sequential ultracentrifugation in a Beckman XL70 ultracentrifuge using a 70.1 Ti rotor (Beckman, CA, USA) at 105,000 g for 24 h at 10 °C according to the method of Schumaker et al. [17]. After the ultracentrifugation, HDL particles were extensively dialysed against phosphate-buffered saline (PBS; pH 7.4) at 4 °C in the dark, stored at 4 °C and used within 3 days for antioxidative activity assays and 10 days for cellular cholesterol efflux tests. During cryopreservation, sucrose solution (0.6% w/v) was used as a preservative.

Total protein (TP), TC, free cholesterol (FC), phospholipid (PL) and TG contents of isolated HDL were determined using commercial kits. CE was calculated by multiplying the difference between TC and FC concentrations by 1.67. Total lipoprotein mass was calculated as the sum of all their chemical components. CVs for these determinations were below 2.3%.

2.5. HDL antioxidative activity

HDL antioxidative activity was assessed towards a reference LDL isolated from a healthy normolipemic control subject [15] in a subgroup of 9 randomly selected IDA patients and their age-matched controls. Antioxidative activity was calculated as the decrease in the propagation rate of LDL oxidation in presence of HDL and expressed as percentage of LDL oxidized alone. Intra and inter-assay CV were 5.3% and 6.0%, respectively.

2.6. HDL-mediated cellular cholesterol efflux

HDL-mediated cellular cholesterol efflux was characterized in HDL particles from a subgroup of 9 randomly selected patients with IDA and their age-matched controls employing a human THP-1 monocytic cell system (ATCC). Assays were performed as previously described [18]. Specific cholesterol efflux was determined by subtracting non-specific cholesterol efflux occurring in the absence of cholesterol acceptors. Assays were performed in triplicate for each sample. Intra- and inter-assay CV were 2.0 and 2.3%, respectively [18].

To test the impact of iron deficiency on cellular cholesterol efflux, human macrophages were pre-treated with 50 and 100 μM of the iron chelator, deferoxamine (DFO, Desferal®, Novartis, Switzerland), and with 100 μM DFO + 50 μM $FeCl_3$ for 8 h. A control of PBS was run in parallel as a blank. After these procedures, incubations with the [^{3}H]cholesterol-labeled acetylated LDL were carried out. In all these tests, cellular cholesterol efflux was assayed towards a reference HDL (15 μg HDL-PL/ml) isolated from one healthy normolipemic subject. Each condition was assayed by triplicate. Results were expressed as percentage of a control incubation with PBS only.

2.7. Statistical analyses

Distribution of all variables was analyzed for normality using the Shapiro–Wilks test. Mean ± SD was used to describe normally distributed variables and median (interquartile range) for non-Gaussian distributed variables. Differences in normally-distributed variables were analyzed using Student's t-test. For skewed variables, the non-parametric Mann–Whitney U test was employed. In order to compare differences between cell treatments, ANOVA with Tukey's *post-hoc* test was employed. Differences in dichotomous variables were analyzed by Fisher's exact test. In the follow-up study, paired tests were employed to evaluate differences. For correlations, Pearson and Spearman coefficients were calculated when appropriate. In order to evaluate the variables associated with ARE activity multiple linear regression was employed. The dependent variable was ARE activity and the independent variables were: age, BMI, Hb concentration, HDL-C, ferritin and CETP activity. Skewed variables were normalized by log-transformation previous to their inclusion in the linear regression model. Tests were considered statistically significant at $p < 0.05$. The statistical softwares employed were INFOSTAT® (University of Córdoba, Córdoba, Argentina) and SPSS® 17.0 (Chicago, Illinois, USA).

3. Results

3.1. Case-control study. Evaluation of metabolic and HDL characteristics

Patients with IDA were comparable in age, BMI, prevalence of overweight/obesity and menopause status with the controls (Table 1). Only two patients showed hypertension and none of the controls. As expected, IDA patients exhibited reduced values of Hb, hematocrit, hematimetric indexes and iron metabolism markers compatible with the diagnosis. Furthermore, patients presented elevated platelet and monocyte counts relative to controls (0.5 ± 0.2 *vs.* 0.4 ± 0.1 10^3 cells/ml, $p < 0.001$). Conversely, glucose metabolism indicators were similar between patients and controls.

Lipoprotein profile of IDA patients was characterized by elevated TG levels and increased CETP activity relative to controls (Table 2). On the other hand, TC and HDL-C levels were decreased in IDA patients *vs.* controls. It is important to note that most of the results were comprised within the reference values. CETP activity was inversely correlated with HDL-C ($r = -0.45$, $p < 0.01$) and directly correlated with TG levels ($r = 0.49$, $p < 0.005$).

The analysis of the chemical composition of HDL particles from IDA patients and controls is shown in Fig. 1. HDL from IDA patients revealed higher TG content than HDL from controls (Fig. 1). Interestingly, CETP activity was positively correlated with the HDL-TG content ($r = 0.55$, $p < 0.01$).

Table 1
Clinical and general biochemical characteristics from IDA patients and controls.

	IDA patients (n = 18)	Control subjects (n = 18)	p
Age (years)	44 ± 8	44 ± 8	0.969
BMI (Kg/m^2)	24 ± 4	23 ± 2	0.692
Overweight/obesity (n)	4/0	3/0	0.708
Menopause (yes/no)	4/14	4/14	0.999
Hct (%)	30 ± 3	40 ± 2	<0.001
Hb (g/dl)	9.7 (8.8–10.3)	13.1 (12.5–13.8)	<0.001
MCV (fl)	72 (68–78)	89 (86–92)	<0.001
RDW (%)	17.9 (15.2–18.7)	13.1 (12.9–14.0)	<0.001
WBC (10^3 cells/ml)	5.7 (4.7–6.7)	6.4 (5.3–7.0)	0.537
Platelets (10^3 cells/ml)	322 ± 69	275 ± 59	0.018
Tf saturation (%)	6 (3–8)	28 (19–37)	<0.001
Ferritin (μg/l)	5.6 (4.3–9.0)	39.3 (19.0–68.7)	<0.001
Glucose (mg/dl)	88 ± 8	84 ± 13	0.434
Insulin (μU/ml)	6.3 (3.0–7.8)	4.2 (2.4–6.5)	0.111
HOMA-IR	1.3 (0.7–1.8)	1.0 (0.5–1.4)	0.138

IDA, iron deficiency anemia; BMI, body mass index; Hct, hematocrit; Hb, hemoglobin; MCV, mean corpuscular volume RDW, red blood cell distribution width; WBC, white blood cells; Tf, transferrin; HOMA, *homeostasis model assessment*. Results are expressed as mean ± SD or median (Q1–Q3) for normal or skewed data, respectively.

Table 2
Lipid and lipoprotein profile from IDA patients and controls.

	IDA patients (n = 18)	Control subjects (n = 18)	p
TG (mg/dl)	89 (74–108)	61 (52–71)	<0.001
TC (mg/dl)	172 ± 31	192 ± 36	0.049
VLDL-C (mg/dl)	17(12–22)	14(10–18)	0.160
LDL-C (mg/dl)	106 ± 27	114 ± 28	0.254
HDL-C (mg/dl)	52 ± 15	64 ± 16	0.013
ApoB (mg/dl)	83 ± 20	80 ± 21	0.655
Apo A-I (mg/dl)	149 (128–173)	156 (138–204)	0.095
CETP activity (%/ml.h)	176 ± 35	128 ± 26	<0.001

IDA, iron deficiency anemia; TG, triglycerides; TC, total cholesterol; VLDL, very low density lipoprotein; LDL, low density lipoprotein; HDL, high density lipoprotein; apo, apolipoprotein; CETP, cholesteryl ester transfer protein. Results are expressed as mean ± SD or median (Q1–Q3) for normal or skewed data, respectively.

Antioxidative activity of HDL and its capacity to promote cellular cholesterol efflux were studied in a subgroup of 9 randomly selected patients and their age-matched controls. HDL from IDA patients displayed reduced antioxidative capacity *vs.* that from control subjects (HDL inhibition of propagation rate of LDL oxidation, 50 ± 14 *vs.* 65 ± 14% of LDL oxidized alone, respectively, $p < 0.05$). The antioxidative activity of HDL was positively correlated with Hb concentration ($r = 0.55$, $p < 0.001$) and transferrin saturation ($r = 0.70$, $p < 0.001$), attesting for its relationship with IDA.

When HDL–mediated cellular cholesterol efflux was evaluated, HDL particles from IDA women and controls showed similar activity (4.3 ± 1.0 *vs.* 4.9 ± 0.8%, respectively, $p > 0.05$). However, cellular iron deficiency, induced by a pre-incubation with DFO, provoked a significant reduction in the cellular cholesterol efflux from macrophages to HDL. Such effect was not observed when DFO was co-incubated with 50 μM $FeCl_3$ (Fig. 2).

Results of inflammatory and oxidative stress markers are shown in Table 3. While hsCRP, MDA and PON activity were not different between patients and controls, Lp-PLA_2 activity tended to be increased in IDA women ($p = 0.057$). On the other hand and consistent with previous results [8,11], ARE activity was significantly reduced in IDA patients in comparison with controls.

Multiple linear regression revealed that nearly half of the variation of ARE activity was attributed to variations in HDL-C levels in a model adjusted for age, BMI, Hb, ferritin concentration and CETP activity (Supp. Table 1). In addition, ARE activity was positively correlated with HDL antioxidative activity ($r = 0.60$, $p < 0.05$) and negatively with MDA levels ($r = -0.37$, $p < 0.05$).

3.2. Longitudinal study. Subacute effects of intravenous iron administration

Sixteen patients (aged 45 ± 12 years) were evaluated before and 72 h after the intravenous administration of iron sucrose. Six patients were hypertensive. Complete blood count, iron and glucose

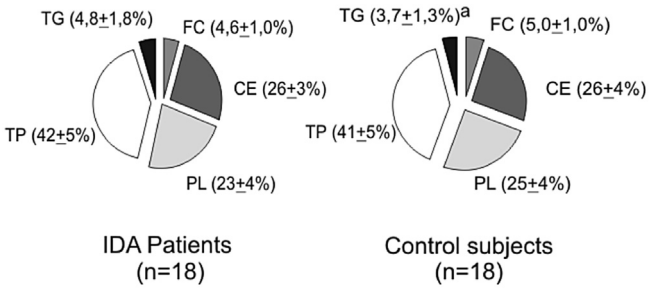

Fig. 1. HDL chemical composition from IDA patients and control subjects. FC, free cholesterol; CE, cholesteryl ester; TG, triglyceride; PL, phospholipid; TP, total protein. Data are expressed as means ± SD. [a]p<0.05 vs. IDA.

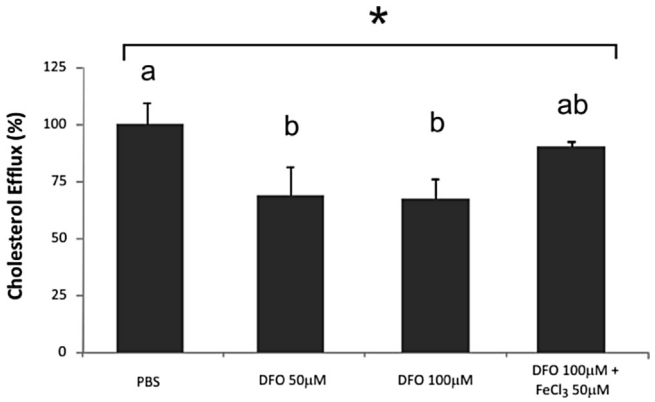

Fig. 2. Effect of iron deficiency, induced by the iron chelator, deferoxamine, on cellular cholesterol efflux. DFO, deferoxamine. Results were expressed as a percentage of cholesterol efflux from PBS-treated cells. The bars represent mean ± standard error of mean (SEM) of triplicates. Differences were tested by ANOVA using Tukey's *post-hoc* test. *p < 0.05, different letters indicate significantly dissimilar groups.

Table 3
Inflammatory and oxidative stress biomarkers from IDA patients and controls.

	IDA patients (n = 18)	Control subjects (n = 18)	p
hsCRP (mg/l)	0.7 (0.5–2.0)	0.8 (0.4–1.4)	0.338
Lp-PLA_2 (μmol/ml.h)	8.8 ± 2.2	7.4 ± 1.8	0.057
MDA (μM)	2.8 ± 0.5	2.7 ± 0.4	0.638
PON (nmol/ml.min)	232 (128–329)	240 (175–349)	0.510
ARE (μmol/ml.min)	124 ± 28	147 ± 28	0.029

IDA, iron deficiency anemia; hsCRP, high sensitivity C-reactive protein; Lp-PLA_2, lipoprotein-associated phospholipase A_2; MDA, malondialdehyde; PON, paraoxonase activity of the enzyme paraoxonase-1; ARE, arylesterase activity of the enzyme paraoxonase-1. Results are expressed as mean ± SD or median (Q1–Q3) for normal or skewed data, respectively.

metabolism markers and lipoprotein profile from baseline and follow-up samples are shown in Table 4. As expected, transferrin saturation and ferritin concentration significantly increased after iron administration. In addition, a slight but significant reduction in insulin concentrations and HOMA-IR was observed. No changes were evidenced in the lipoprotein profile.

On the other hand, though no significant differences were found in hsCRP, PON and Lp-PLA_2 levels (Supp. Table 2), the intravenous administration of iron was associated with a reduction in MDA levels and an increase in ARE activity (Fig. 3).

4. Discussion

Oxidative stress has been one of the hypotheses proposed to explain the link between IDA and increased risk of CVD [5]. In general, the main support to this notion comes from studies in which IDA patients showed reduced activity of antioxidant enzymes which depend on iron [12,19]. The present manuscript further extends such results by revealing alterations in HDL antioxidative capacity. In addition, IDA was associated with an altered HDL chemical composition and impaired ARE activity, an HDL-bound antioxidant enzyme. The observation that ARE activity was correlated with MDA levels suggests a role for HDL in the balance between prooxidants and antioxidants in IDA. Indeed, the follow-up study showed an increase in ARE activity and a reduction in MDA levels 72 h after an infusion of iron sucrose. Therefore, adequate iron levels would be necessary to maintain HDL antioxidative function as it is the case for other enzymatic antioxidant systems that depend on iron.

In agreement with previous studies [8,11,20], women with IDA showed elevated TG and reduced HDL-C concentrations as compared to controls. Nonetheless, lipid levels among IDA patients were mostly within the reference range. The observed differences in the lipoprotein profile between patients and controls appear to be unrelated to insulin resistance, as glucose metabolism markers were similar between the groups. Such observation agrees with results of previous studies [21,22]. Then, the differences in the lipoprotein profile observed could be associated with a delayed catabolism of TG-rich lipoproteins, as well as an accelerated hepatic VLDL production [23,24]. In this context, increased TG levels could induce an increase in CETP activity, which should eventually alter not only VLDL but also HDL particles. Indeed, HDL from IDA patients presented elevated TG content relative to controls. Such compositional alteration was significantly associated with CETP activity. Of importance, no differences were observed on the food frequency questionnaires administered to the subjects in the case control study. On the other hand, intravenous iron administration was not followed by modifications in the lipoprotein profile, as it was observed in another study [20]. Therefore, beneficial changes in the lipoprotein profile might be noticed only after anemia is corrected.

Iron deficiency induced *in-vitro* by DFO was associated with a reduction in cellular cholesterol efflux to a reference HDL. In agreement, a relationship between iron metabolism and cellular cholesterol metabolism has been pointed out by others [25,26]. The DFO dose used in the present study was similar to other studies in which proinflammatory actions were also attributed to iron deficiency [9,10]. In particular, Fan et al. [10] described an enhanced activation of the proinflammatory transcription factor, NF-κβ, in iron-deficient macrophages. Then, as a consequence of proinflammatory signaling, cholesterol efflux might be impaired in iron-deficient macrophages [27]. In the context of IDA, reduced capacity of macrophages to donate cholesterol to HDL may contribute to low HDL-C levels and increased CVD risk.

The link between IDA and deficient antioxidative activity of HDL in the present study can be attributed, at least in part, to low ARE activity. Indeed, women with IDA presented reduced ARE activity

Table 4
Subacute effects of intravenous iron administration on hematological parameters, iron metabolism indicators and lipoprotein profile.

(n = 16)	Pre	Post 72hs	Paired differences [median (CI95)]	p
Hct (%)	29 ± 3	31 ± 3	0.9 (−0.2–2.0)	0.099
Hb (g/dl)	9.7 ± 1.4	10.1 ± 1.2	0.3 (−0.1–0.7)	0.071
MCV (fl)	74 ± 8	75 ± 7	0.6 (−0.6–1.8)	0.282
RDW (%)	17.5 (15.6–19.0)	17.9 (15.8–19.3)	0.3 (−0.4–1.0)	0.223
WBC (10^3 cells/ml)	6.1 ± 1.9	6.0 ± 1.8	−0.1 (−0.8–0.6)	0.736
Platelets (10^3 cells/ml)	320 ± 96	296 ± 76	−10 (−44–23)	0.508
Tf saturation(%)	7 ± 3	18 ± 8	11 (6–15)	<0.001
Ferritin (ng/ml)	5.7 (4.3–10.3)	105.0 (70.0–213.3)	127 (74–180)	<0.001
Glucose (mg/dl)	92 ± 11	86 ± 12	−6 (−14–2)	0.732
Insulin (μU/ml)	6.3 (3.0–8.3)	5.5 (2.8–7.5)	−2.1 [−2.7–(−0.4)]	0.0256
HOMA-IR	1.4 (0.7–2.1)	1.1 (0.6–1.5)	−0.4 [−0.7–(−0.1)]	0.019
TG (mg/dl)	91 (74–108)	95 (72–134)	6 (−15–28)	0.985
TC (mg/dl)	167 ± 32	169 ± 29	1 (−11–13)	0.821
VLDL-C (mg/dl)	15 ± 6	15 ± 8	0.5 (−9.6–10.6)	0.881
LDL-C (mg/dl)	102 ± 26	105 ± 29	3 (−6–12)	0.460
HDL-C (mg/dl)	51 ± 14	49 ± 11	−2 (−10–6)	0.608
Apo B (mg/dl)	78 ± 20	80 ± 21	2 (−4–8)	0.441
Apo A-I (mg/dl)	153 ± 29	153 ± 22	2 (−19–23)	0.830
CETP (%/ml.h)	196 ± 21	221 ± 60	25 (−15–64)	0.191

IDA, iron deficiency anemia; CI, confidence interval; BMI, body mass index; Hct, hematocrit; Hb, hemoglobin; MCV, mean corpuscular volume; RDW, red blood cell distribution width; WBC, White blood cells; Tf, transferrin; HOMA, *homeostasis model assessment*. TG, triglyceride; TC, total cholesterol; VLDL, very low density lipoprotein; LDL, low-density lipoprotein; HDL, high-density lipoprotein; apo, apolipoprotein; CETP, cholesteryl ester transfer protein. Results are expressed as mean ± SD or median (Q1–Q3) for normal or skewed data, respectively.

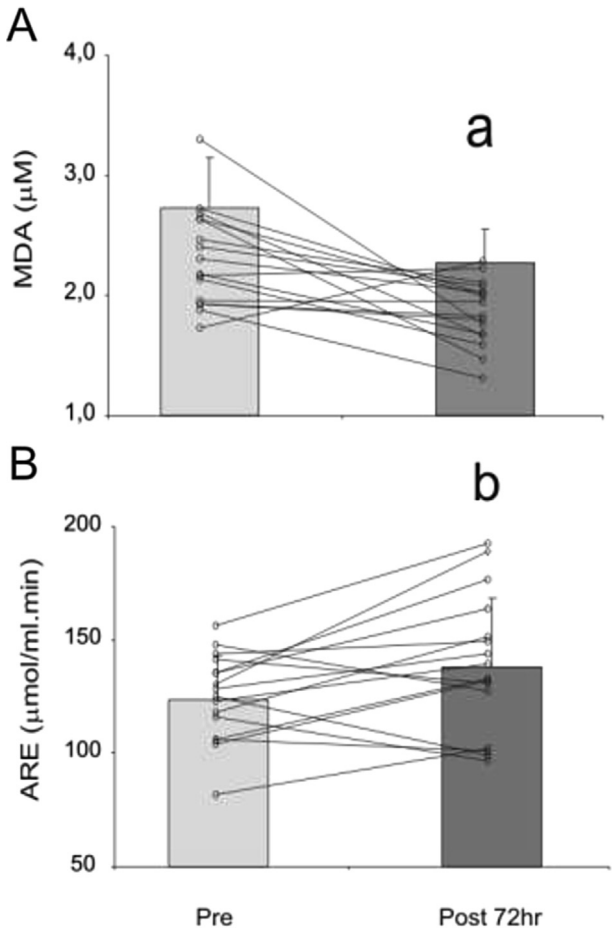

Fig. 3. MDA levels (Panel A) and ARE activity (Panel B) in IDA patients 72 h after a 300 mg/2 h infusion of sucrose iron (n = 16). MDA, malondialdehyde, ARE, arylesterase activity of the enzyme paraoxonase-1. The connected lines show individual values and the bars represent the group mean and SD. [a]p<0.005; [b]p < 0.05.

and the administration of iron led to a significant increase in this activity. In addition, decreased antioxidative activity of HDL could be related to an altered association between transferrin and HDL as reported by Kunitake et al. [28]. Unfortunately, we could not study whether iron administration was able to fully restore HDL antioxidative activity due to insufficient volume of serum for sequential ultracentrifugation in the follow-up study. However, it is important to note that HDL antioxidative activity was positively correlated with Hb levels. Such result suggests that correction of iron deficiency and of anemia should lead to improvement of HDL antioxidative activity.

The impact of intravenous iron on oxidative stress and inflammatory markers was evaluated by several studies in patients with kidney disease and IDA [19,29]. In general, these studies showed an increase in MDA levels and other markers of oxidative stress 1–4 h after the infusion of intravenous iron. In this regard, it seems that differences in the evaluation time after the iron administration could be responsible for the differences between those studies and ours. Most likely, intravenous iron triggers oxidative stress during the first hours after the infusion [19,29]. However, due to the increased availability of iron and as a response to the initial oxidative stress caused by the rapid increase in iron concentration, a mobilization of antioxidant enzymes might occur. Indeed, ARE activity increased and MDA levels decreased 72 h after the infusion of intravenous iron. Pai et al. and Ozkurt et al. obtained similar results when evaluating patients with chronic kidney disease 3 and 7 days, respectively, after the administration of intravenous iron [30,31]. Regarding inflammatory status, no significant differences were observed in the inflammatory markers measured. Such results confirm the security profile of the formulation of sucrose iron used in the protocol. In women with a more severe anemia, improvement in inflammatory markers might be detected after achievement of normal Hb levels.

In conclusion, women with IDA presented HDL particles enriched in TG, with a diminished capacity to inhibit LDL oxidation. The administration of intravenous iron led to a decrease in oxidative stress markers and an increase in the HDL-bound antioxidant enzyme paraoxonase-1. IDA is characterised by elevated oxidative stress and functionally deficient HDL particles. It remains to be determined if such alterations suffice to impair endothelial function in IDA.

Conflict of interest

The authors have no conflict of interests.

Authors' contribution

TM, CD, WT, EB and ML evaluated the activity of the lipoprotein-associated enzymes, performed the HDL ultracentrifugation and characterized their chemical composition and functionality. TM, MM done the statistical analyses and partially wrote the first draft of the manuscript. MSS, PS and LB carried out the blood extractions, performed the general biochemical determinations and helped to carry out the recruitment. JA was responsible for the recruitment of both patients and controls. TM, JC, AK, and FB conceived the study, and participated in its design and coordination and wrote part of the first draft of the manuscript. All authors read and approved the final manuscript.

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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.clnu.2016.02.003.

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