Persistence of an atherogenic lipid profile after treatment of acute infection with brucella

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Abstract Serum lipid changes during infection may be associated with atherogenesis. No data are available on the effect of Brucellosis on lipids. Lipid parameters were determined in 28 patients with Brucellosis on admission and 4 months following treatment and were compared with 24 matched controls. Fasting levels of total cholesterol (TC), HDL-cholesterol (HDL-C), triglycerides, apolipoproteins (Apo) A , B, E CII, and CIII, and oxidized LDL (oxLDL) were measured. Activities of serum cholesterol ester transfer protein (CETP), paraoxonase 1 (PON1), and lipoprotein-associated phospholipase A_2 (Lp-PLA_2) and levels of cytokines [interleukins (IL)-1β, IL-6, and tumor necrosis factor (TNFa)] were also determined. On admission, patients compared with controls had *1*) lower levels of TC, HDL-C, LDL-cholesterol (LDL-C), ApoB, ApoAI, and ApoCIII and higher LDL-C/HDL-C and ApoB/ApoAI ratios; *2*) higher levels of IL-1b, IL-6, and TNFa; *3*) similar ApoCII and oxLDL levels and Lp-PLA_2 activity, lower PON1, and higher CETP activity; and *4*) higher small dense LDL-C concentration. Four months later, increases in TC, HDL-C, LDL-C, ApoB, ApoAI, and ApoCIII levels, ApoB/ApoAI ratio, and PON1 activity were noticed compared with baseline, whereas CETP activity decreased. LDL-C/HDL-C ratio, ApoCII, and oxLDL levels, Lp-PLA_2 activity, and small dense LDL-C concentration were not altered. Brucella infection is associated with an atherogenic lipid profile that is not fully restored 4 months following treatment.—Apostolou, F., I. F. Gazi, A. Kostoula, C. C. Tellis, A. D. Tselepis, M. Elisaf, and E. N. Liberopoulos. **Persistence of an atherogenic lipid profile after treatment of acute infection with brucella.** ***J. Lipid Res.*** **2009.** 50: **2532–2539.**

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There is increasing evidence that a link exists between infection/inflammation and atherosclerosis (1). Infections with chlamydia pneumoniae, cytomegalovirus, herpes simplex virus, helicobacter pylori, as well as periodontitis have been studied (2–4). On the contrary, other studies have disputed the causal role of infectious agents in atherogenesis (5–7).

Current evidence suggests that atherosclerosis develops as a response to inflammatory stimulus. Therefore, common or uncommon infections could represent a risk factor. Mechanisms that may be implicated in the atherogenesis caused by infectious agents include local increase of proinflammatory cells, local effusion of endotoxins, autoimmune reaction, systemic cytokine release, and changes in lipid metabolism (8).

Infection and inflammation cause similar cytokine-induced changes in lipid and lipoprotein metabolism (9). These include reductions in serum levels of total cholesterol (TC), HDL-cholesterol (HDL-C), LDL-cholesterol (LDL-C), apolipoproteins (Apo) AI and B, and lipoprotein (a) [Lp(a)] and increases in triglyceride (TG) and ApoE concentrations (9–14).

Current evidence suggests that the host response to infection and inflammation increases oxidized lipids in serum and induces LDL oxidation in vivo. Oxidative modification of LDL is an important event in the development of atherosclerosis (15). In addition, the cholesterol ester transfer protein (CETP) plays a central role in HDL metabolism and the regulation of HDL-C levels in serum. High levels of CETP activity lead to a reduction in HDL-C levels and an atherogenic lipoprotein profile (16).

Platelet-activating factor (PAF) is a potent pro-inflammatory phospholipid produced by activated platelets,

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Abbreviations: Apo, apolipoprotein; CETP, cholesterol ester transfer protein; CRP, C-reactive protein; CV, coefficients of variation; HDL-C, HDL-cholesterol; IgA, immunoglobulin; IL, interleukin; LDL-C, LDL-cholesterol; Lp(a), lipoprotein (a); Lp-PLA_2, lipoprotein-associated phospholipase A_2; LPS, lipopolysaccharide; oxLDL, oxidized LDL; PAF, platelet-activating factor; PON1, paraoxonase 1; sdLDL, small dense LDL; TC, total cholesterol; TG, triglyceride; TNF, tumor necrosis factor.

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leukocytes, and endothelial cells. PAF is degraded by PAF acetylhydrolase, also known as lipoprotein-associated phospholipase A_2 (Lp-PLA_2), an enzyme that catalyzes the hydrolysis of the acetyl group at the *sn*-2 position of PAF (17). Lp-PLA_2 also hydrolyzes phosphatidylcholine, resulting in the production of lysophosphatidylcholine, a molecule that may mediate various biologic effects of oxidized LDL (oxLDL) (17). Lp-PLA_2 is mainly distributed on the LDL subclasses (80%), while the remaining is found on HDL (HDL-Lp-PLA_2) (17). Studies have shown that an increase in Lp-PLA_2 mass or activity represents an independent risk factor for the development of atherosclerosis (18, 19). On the other hand, HDL-Lp-PLA_2 may exhibit antiatherogenic properties (17). Various changes in Lp-PLA_2 plasma activity in response to infection and inflammation have been reported among different animal species (20) and in population-based studies (21, 22). Therefore, infection-induced atherogenesis could, at least in part, be mediated by alterations of Lp-PLA_2 activity.

Paraoxonase 1 (PON1) plays an important role in HDL-mediated anti-atherogenic action. PON1 is an esterase exclusively associated with HDL in plasma and catalyzes the hydrolysis of phospholipid hydroperoxides and cholesteryl ester hydroperoxides formed during LDL oxidation (23). During infection and inflammation, serum PON1 activity decreases and acute-phase HDL is unable to protect LDL against oxidation (24–26).

LDLs consist of a heterogeneous family of particles with different size, composition, and density (27, 28). Several studies have shown that small dense LDL (sdLDL) particles are more atherogenic (18, 29, 30) compared with large buoyant ones. The effects of inflammation on the distribution of LDL subclasses have been investigated (9, 31). An increase in sdLDL particles in response to infection has been reported in some studies (2, 32).

Brucellosis is a zoonosis most commonly encountered in rural Mediterranean areas (33). The vast majority of cases are attributed to the subtype *Brucella melitensis*. The effect of Brucellosis on lipid parameters is currently unknown. We undertook this study to evaluate the possible quantitative and qualitative effects of acute Brucellosis on lipoprotein metabolism.

MATERIALS AND METHODS

Study population

Twenty-eight consecutive patients who were admitted, diagnosed, and treated for acute Brucellosis in the Department of Internal Medicine, University Hospital of Ioannina, Greece, between January 2003 and September 2005 were included. No patient was receiving any hypolipidaemic agents or had any clinical or laboratory evidence of any disease known to affect lipid metabolism, such as neoplasia, renal or liver dysfunction, and hypo- or hyperthyroidism.

All patients were examined on admission and 4 months following successful treatment. No change in the patient dietary habits and body weight was recorded during follow-up.

The diagnosis of acute Brucella infection was established by the presence of specific IgM antibodies against Brucella as determined by ELISA.

Patients that required extended treatment were excluded from the study. Thus, patients with Brucellosis complicated with endocarditis, meningitis, spondylitis, or localized abscesses were not included in this study. Moreover, patients with chronic disease [symptoms for more than 12 months or the appearance of class A immunoglobulins (IgA) in conjunction with class G (IgG) for longer than 6 months before the admission] were excluded. Patients with evidence of malnutrition (serum albumin levels <3.6 g/dl) were also excluded. Patient nutritional status did not change during observation (based on questions about quantity and quality of food intake). All patients received doxycycline 100 mg orally twice daily and rifampin 600 mg orally daily for 6 weeks.

The control group consisted of 24 age- and sex-matched healthy individuals who visited our Outpatient Clinic for a regular checkup. The control group had no detectable antibodies to Brucella. All subjects gave informed consent for inclusion in this study, and the study protocol was approved by our Institutional Ethics Committee.

Biochemical measurements

Fasting serum levels of TC, HDL-C, and TG were determined enzymatically on an Olympus AU600 Clinical Chemistry analyzer (Olympus Diagnostica, Hamburg, Germany). LDL-C was calculated using the Friedewald formula. None of the patients had TG levels exceeding 350 mg/dl. Apo's AI, B, and E as well as Lp(a) levels were measured with a Behring Nephelometer BN100 using reagents (antibodies and calibrators) from Date Behring Holding (Liederbach, Germany). ApoCII and ApoCIII were determined by an immunoturbidimetric assay provided by Kamiya Biomedical (Seattle, WA).

Plasma levels of oxLDL were measured by a competitive ELISA using a specific murine monoclonar antibody (4E6) according to the instructions provided by the manufacturer (Mercodia, Uppsala, Sweden). The specificity of this method was studied by performing the assay in five different plasma samples in which 5 or 15 ng of protein of native LDL or OxLDL was added exogenously. Intra-assay and inter-assay coefficients of variation of the assay were 6.0% and 7.0%, respectively (34).

CETP activity was measured by a fluorometric assay, using a commercially available kit (Roar Biomedical, New York, NY). Briefly, 2 μl of plasma diluted 1:1 with sample buffer (10 mmol/l Tris, 150 mmol/l NaCl, and 2 mmol/l EDTA, pH 7.4) were used as the source of CETP. The assay was performed for 1 h at 37°C, and the CETP activity was determined by the increase in fluorescence intensity measured in a fluorescence spectrometer at an excitation wavelength of 465 nm and emission wavelength of 535 nm (35, 36).

Lp-PLA_2 activity was measured by the trichloroacetic acid precipitation procedure using 1-*O*-hexadecyl-2-[^{3}H-acetyl] *sn*-glycero-3-phosphocholine (10 Ci/mmol; DuPont-New England Nuclear, Boston, MA) as a substrate at a final concentration of 100 μM. Fifty microliters of either serum diluted 1:50, v/v, with HEPES buffer, pH 7.4, or the HDL-containing supernatant after treatment of serum with magnesium chloride-dextran sulfate (HDL-rich serum) (diluted 1:3, v/v, with HEPES) were mixed with HEPES in a final volume of 90 μl and used as the source of the enzyme. The reaction was performed for 10 min at 37°C, and Lp-PLA_2 activity was expressed as nmol PAF degraded per ml of serum per min (37). Reproducibility of Lp-PLA_2 assays was determined by intra-assay determination of coefficients of variation (CVs). The CVs for Lp-PLA_2 assays ranged between 4% and 5%.

We measured serum PON1 hydrolyzing activity against paraoxon (PON1 (paraoxonase)] and phenyl acetate [PON1 (arylesterase)]. Both PON1 (paraoxonase) and PON1 (arylesterase) activities in serum were determined in the presence of 2 mM Ca^{2+} in 100 mM Tris-HCl buffer (pH 8.0) for paraoxon as

a substrate and in 20 mM Tris-HCl buffer (pH 8.0) for phenylacetate as a substrate, respectively, as previously described (38).

Cytokine [interleukins (IL)-1β and IL-6 and tumor necrosis factor (TNFa)] were determined by ELISA using a commercially available immunoassay kit (Quantikine; R and D Systems, Minneapolis, MN). Each sample was measured in duplicate with appropriate sensitivities for IL-1b (<0.1 pg/ml), IL-6 (<0.7 pg/ml), and TNFa (<4.4 pg/ml). The median and mean intra-assay and inter-assay CVs were <10% for all the above measurements. C-reactive protein (CRP) levels were measured by an immunoturbidimetric assay (Roche Diagnostics). The ELISA employed (Serion ELISA Classic Brucella IgG/IgM/IgA; Institut Virion/Serion, Germany) detected specific IgG, IgM, and IgA anti-Brucella antibodies and was used in accordance with the manufacturer's instructions.

LDL subclass analysis was performed electrophoretically using high-resolution 3% polyacrylamide gel tubes and the Lipoprint LDL System (Quantimetrix) according to the manufacturer's instructions (39–41). Briefly, 25 μl of sample was mixed with 200 μl of Lipoprint Loading Gel and placed upon the upper part of the 3% polyacrylamide gel. After 30 min of photopolymerization at room temperature, electrophoresis was performed for 60 min with 3 mA for each gel tube. Each electrophoresis chamber involved two quality controls (sample provided by the manufacturer). For quantification, scanning was performed with a ScanMaker 8700 digital scanner (Mikrotek) and iMac personal computer (Apple Computer). After scanning, electrophoretic mobility (Rf) and the area under the curve were calculated qualitatively and quantitatively with the Lipoprint LDL system Template and the Lipoware software (Quantimetrix, Redondo Beach, CA), respectively. The LDL subfraction was calculated using the Rf between the VLDL fraction (Rf 0.0) and the HDL fraction (Rf 1.0). LDL is distributed from Rf 0.32 to 0.64 as seven bands, whose Rfs are 0.32, 0.38, 0.45, 0.51, 0.56, 0.6, and 0.64 (LDL1 to LDL7, respectively). LDL1 and LDL2 are defined as large, buoyant LDL and LDL3 to LDL7 are defined as sdLDL. The cholesterol concentration of each LDL subfraction is determined by multiplying the relative area (area under the curve) of each subfraction by the TC concentration of the sample (the TC concentration of the sample is measured independently). Moreover, the Lipoprint LDL System provides a mean LDL particle size (nm).

Statistical analysis

Data are presented as mean (SD) except for non-normal distributed variables, which are presented as median (range). The Kolmogorov-Smirnov test was used to evaluate whether each variable followed a Gaussian distribution. The relationships among study variables were investigated using the Pearson product moment correlation coefficient (r), whereas correlations including at least one non-normal variable were performed using Spearman correlation coefficient (rho). Multivariate analysis was used to determine which factors were independently related to alterations of study parameters. Significance was set at $P < 0.05$.

Paired Student's t-test (or Wilcoxon's Rank test) was applied for comparisons between study parameters before and after treatment. Unpaired t-test (or Mann-Whitney test as appropriate) was used to compare data between patients and controls. All analyses were carried out with SPSS 13.0 (SPSS, 1989–2004, Chicago, IL).

RESULTS

Characteristics of study population

All patients (18 male and 10 female, mean age 52.9 ± 14.6 years) had a history of occupational exposure to Brucella. They were admitted to hospital because of fever, myalgias, arthralgias, or constitutional symptoms, such as malaise and weakness. Twenty-four age- and sex-matched healthy individuals were included. The clinical and biochemical characteristics of study participants are shown in **Table 1**.

Serum lipid profile

On admission, patients had significantly lower levels of TC, HDL-C, LDL-C, ApoB, and ApoAI compared with controls, while there were no significant differences in TGs, ApoE, and Lp(a) levels (Table 1). Moreover, ApoB/ApoAI and LDL-C/HDL-C ratios were significantly higher in patients on admission compared with controls (Table 1). ApoCII levels did not differ between Brucellosis patients at study entry and controls, whereas levels of ApoCIII were lower in patients on admission compared with controls (Table 1). No difference was observed in oxLDL levels and oxLDL/ApoB ratio between patients on admission and controls (Table 1). CETP activity was higher in patients on admission compared with controls (Table 1).

On admission, a significant negative correlation between TC, HDL-C, LDL-C, ApoB, and ApoAI with IL-6 levels was observed ($r = -0.40$, -0.36, -0.38, -0.39, and -0.42, respectively, P for all <0.05) but not with other cytokines. We also observed a significant negative correlation ($r = -0.49$, $P < 0.05$) between ApoAI and CRP as well as a significant positive correlation between TGs and CRP ($r = 0.42$, $P < 0.05$).

Four months following successful treatment, significant increases in levels of TC, HDL-C, LDL-C, ApoB, and ApoAI were noticed (Table 1). On the other hand, levels of TGs, ApoB, ApoE, and Lp(a) as well as the LDL-C/HDL-C ratio were not significantly altered. Also, the ApoB/ApoAI ratio significantly decreased (Table 1). ApoCII levels remained unchanged following treatment, whereas a significant increase in ApoCIII levels was noticed (Table 1). In addition, no difference in oxLDL levels and oxLDL/ApoB ratio before and after treatment was observed (Table 1). CETP activity decreased after treatment compared with values on admission (Table 1). Changes in CETP activity (ΔCETP) were negatively correlated with changes in HDL-C levels (ΔHDL-C) during treatment ($r = -0.42$, $P = 0.02$).

Hematologic response

No significant difference in numbers of total white blood cells as well as individual components (neutrophils, lymphocytes, and monocytes) was noticed either between patients on admission compared with controls or between patients before and after treatment (Table 1).

Lp-PLA$_2$ activity

Total plasma and HDL-associated Lp-PLA$_2$ activity as well as the ratio total plasma Lp-PLA$_2$/ApoB did not differ between patients on admission and controls (**Table 2**).

We studied possible correlations between Lp-PLA$_2$, cytokines, and CRP in patients on admission. Total plasma Lp-PLA$_2$ activity was negatively correlated with IL-6 ($r = -0.45$, $P < 0.05$) and TNFa levels ($r = -0.53$, $P = 0.01$). No significant correlations between HDL-Lp-PLA$_2$ activity and

TABLE 1. Clinical and laboratory characteristics of controls and patients at baseline and 4 months after successful treatment

	Patients with Acute Brucellosis on Admission (n = 28)	Controls (n = 24)	P for the Comparison of Patients on Admission with Controls	Patients with Acute Brucellosis 4 Months after Successful Treatment (n = 28)	P for the Comparison of Patients on Admission with Those 4 Months after Successful Treatment
Age	52.9 ± 14.6	50.6 ± 10.2	NS	–	–
Sex (M/F)	18/10	14/10	NS	–	–
BMI (kg/m^2)	26.1 ± 4.2	26.8 ± 4.5	NS	26.3 ± 4.8	NS
Smokers (yes/no)	10/18	9/16	NS	10/18	NS
T-CHOL (mg/dl)	171 ± 27	221 ± 31	<0.001	211 ± 40	<0.001
HDL-C (mg/dl)	35 ± 8	59 ± 9	<0.001	48 ± 9	<0.001
TG (mg/dl)	122 ± 40	111 ± 41	NS	125 ± 80	NS
LDL-C (mg/dl)	114 ± 28	139 ± 31	<0.05	140 ± 31	0.01
LDL-C/HDL-C	3.4 ± 0.1	2.4 ± 0.7	<0.001	3.0 ± 0.9	NS
ApoAI (mg/dl)	95 ± 19	158 ± 22	<0.001	132 ± 23	<0.001
ApoB (mg/dl)	87 ± 22	98 ± 22	<0.05	96 ± 25	<0.05
ApoB/ApoAI	0.97 ± 0.37	0.60 ± 0.20	<0.001	0.75 ± 0.22	<0.001
ApoE (mg/l)	37 ± 10	42 ± 12	NS	38 ± 12	NS
ApoCII (mg/dl)	3.4 (1.3–7.3)	3.3 (2.5–7.3)	NS	3.6 (1.7–8.4)	NS
ApoCIII (mg/dl)	7.6 ± 1.7	9.7 ± 2.3	<0.05	10.5 ± 3.9	0.02
Lp(a) (mg/dl)	17.1 (2.5–131)	15.0 (3.8–70.0)	NS	18.6 (2.5–67.6)	NS
oxLDL (U/L)	33.6 (20.4–66.8)	34.3 (29.8–60.6)	NS	37.1 (17.3–162.4)	NS
oxLDL/ApoB (U/mg)	0.04 (0.02–0.06)	0.04 (0.02–0.05)	NS	0.04 (0.02–0.06)	NS
CETP (nmol/ml/h)	191 ± 50	175 ± 38	0.04	170 ± 57	0.04
VLDL-C (mg/dl)	32 ± 10	30 ± 13	NS	31 ± 14	NS
Large LDL-C (mg/dl)	86 ± 14	125 ± 13	<0.01	104 ± 20	<0.01
sdLDL-C (mg/dl)	11 ± 4	5 ± 3	<0.05	14 ± 5	NS
LDL particle size (Å)	264 ± 3	270 ± 3	<0.001	263 ± 6	NS

BMI, body mass index; T-CHOL, total cholesterol; VLDL-C, VLDL-cholesterol; sdLDL-C, small dense LDL particle cholesterol; NS, not significant. Values are expressed as mean ± (SD) or median (range).

IL-6, IL-1b, TNFa, and CRP were found. Multiple regression analysis, which included those parameters that were significantly correlated with Lp-PLA$_2$ in univariate analysis, showed that the two significant predictors of total plasma Lp-PLA$_2$ activity were serum levels of TNFa and LDL-C (**Table 3**).

Four months after successful treatment, no change in total Lp-PLA$_2$ activity, HDL-associated Lp-PLA$_2$ activity, and total Lp-PLA$_2$ activity/apoB ratio was noticed (Table 2).

PON1 (paraoxonase) and PON1 (arylesterase) activities

On admission, patients had significantly lower activities of PON1 (paraoxonase) and PON1 (arylesterase) compared with controls (Table 2). Four months after successful treatment, a significant increase in PON1 (arylesterase)

TABLE 2. Lp-PLA$_2$, PON1 (paraoxonase), and PON1 (arylesterase) activities, cytokine levels, and hematologic response in controls and in patients at baseline and 4 months after successful treatment

	Patients with Acute Brucellosis on Admission (n = 28)	Controls (n = 24)	*P* for the Comparison of Patients on Admission with Controls	Patients with Acute Brucellosis 4 Months after Successful Treatment (n = 28)	*P* for the Comparison of Patient Values on Admission with Those 4 Months after Successful Treatment
Total plasma Lp-PLA$_2$ (nmol/min/ml)	50.6 ± 15.7	47.3 ± 13.8	NS	53.6 ± 12.5	NS
HDL-Lp-PLA$_2$ (nmol/min/ml)	2.9 ± 1.9	2.4 ± 0.9	NS	3.2 ± 1.3	NS
Total plasma Lp-PLA$_2$/ApoB (nmol/mg/min)	0.65 ± 0.26	0.58 ± 0.17	NS	0.60 ± 0.18	NS
PON1 (paraoxonase) (U/l)	80.5 (10.0–163.0)	111.5 (23.2–240.7)	<0.001	89.0 (28.0–291.0)	<0.05
PON1 (arylesterase) (U/l)	36.3 ± 7.9	46.2 ± 17.9	<0.05	42.2 ± 7.9	<0.05
CRP (mg/L)	26 (1–134)	3 (1–7)	<0.001	4 (1–66)	<0.001
IL-1β (pg/ml)	4.6 (3.0–152.7)	3.7 (3.0–197.8)	<0.001	3.5 (2.6–33.1)	<0.05
IL-6 (pg/ml)	4.2 (0.3–101.8)	0.8 (0.5–8.6)	<0.01	2.7 (0.4–18.8)	<0.001
TNFa (pg/ml)	3.5 (0.8–35.0)	0.7 (0.1–9.3)	<0.05	3.5 (0.2–44.0)	NS
WBC (/μl)	6,356 ± 2,099	6,123 ± 1,631	NS	6,290 ± 1,389	NS
NEUT (/μl)	3,554 ± 1,516	3,421 ± 1,086	NS	3,188 ± 1,022	NS
LYMPH (/μl)	2,302 ± 1,007	2,177 ± 787	NS	2,522 ± 838	NS
MONO (/μl)	331 ± 136	343 ± 336	NS	296 ± 95	NS

Values are expressed as mean ± (SD) or median (range). WBC, white blood cells; NEUT, neutrophils; LYMPH, lymphocytes; MONO, monocytes; NS, not significant.

TABLE 3. Multiple linear regression analysis for the prediction of total plasma Lp-PLA$_2$ activity in patients on admission

	Total plasma Lp-PLA$_2$		
	β	*P*	
Sex (M/F)	−0.15	0.52	
IL-6	0.03	0.93	
TNFa	−0.91	0.04	$R^2 = 0.83$
CRP	−0.05	0.81	
LDL-C	0.70	0.03	
LDL size	−0.018	0.93	

TABLE 4. Multiple linear regression analysis for the prediction of sdlDL-C concentration and LDL particle size in patients on admission

	sdLDL-C		LDL size	
	Beta	P	Beta	P
Sex (M/F)	−0.55	0.03	0.59	<0.001
TGs	0.59	0.02	−0.62	<0.001
IL-6	0.60	0.05	−0.48	0.05
TNFa	0.33	0.25	−0.1	0.69
CRP	−0.04	0.82	0.06	0.77

and PON1 (paraoxonase) activities was noticed (Table 2). No significant correlations between PON1 (paraoxonase) or PON1 (arylesterase) and any other lipid or cytokine parameter was found (data not shown).

Cytokines and CRP

On admission, patients had significantly higher levels of CRP, IL-1b, IL-6, and TNFa compared with controls (Table 2). Four months after successful treatment, significant decreases in the levels of CRP, IL-1b, and IL-6 were noticed, whereas TNFa levels were not significantly altered (Table 2). We performed again all the analyses after excluding the two patients with partial CRP response (post-treatment CRP values of 66 and 18 mg/L). No essential change in the results was noticed.

LDL subclass analysis

The large LDL-C concentration and mean LDL particle size was lower, whereas sdLDL-C concentration was higher in patients on admission compared with controls (Table 1). VLDL-C levels did not differ between the two groups (Table 1).

Four months after successful treatment, VLDL-C levels, mean LDL particle size, and sdLDL-C concentration were not significantly altered compared with baseline values (Table 1). On the other hand, large LDL-C levels significantly increased (Table 1).

In patients on admission, significant correlations were observed between sdLDL-C concentration and male sex ($r = 0.55$, $P < 0.05$), TGs ($r = 0.35$, $P < 0.05$), levels of IL-6 ($r = 0.39$, $P < 0.05$), and CRP ($r = 0.41$, $P < 0.05$). LDL size was negatively correlated with male sex ($r = -0.58$, $P = 0.01$), TGs ($r = -0.52$, $P < 0.01$), IL-6 ($r = -0.62$, $P < 0.01$), TNFa ($r = -0.49$, $P < 0.01$), and CRP levels ($r = -0.41$, $P < 0.05$). Multiple regression analysis, which included those parameters that were correlated with sdLDL-C and LDL size in univariate analysis, showed that significant predictors of these variables were sex, TGs, and IL-6 concentration (**Table 4**).

DISCUSSION

This study shows for the first time that acute Brucellosis is associated with a shift of serum lipids, lipoproteins, and associated enzymes toward a more atherogenic lipid profile, which is not fully restored 4 months after treatment. Brucellosis is frequent in countries like Greece and others where a large part of the population (mainly in rural areas) is exposed to Brucella. Therefore, if Brucellosis is associated with atherogenic changes in lipid profile that persist following successful treatment, this may be of particular importance from a public health perspective.

Previous studies have shown that lipids are altered in patients with various infections. The majority of studies in humans included patients with sepsis (42, 43). The decrease in cholesterol levels in infections is well known, but the underlying mechanisms remain unclear. Several studies showed that the reduction in cholesterol levels and the alterations in apolipoprotein concentrations were correlated with certain cytokine and acute phase protein levels. Indeed, such correlations were demonstrated in patients with sepsis (44), in neutropenic patients with fever (45), and in normal volunteers after a single intravenous endotoxin injection (46). Moreover, similar associations were noticed in patients with AIDS (47) and in critically ill surgical patients (48).

One explanation for the infection-associated decrease in cholesterol levels is that increased levels of IL-6 stimulate LDL receptor expression in hepatic cells and subsequently lead to increased uptake of LDL particles and decreased LDL-C plasma levels (49). This is consistent with the negative correlation between IL-6 and TC as well as LDL-C levels in our study. Furthermore, cytokines stimulate the displacement of ApoAI by serum amyloid A (an acute-phase protein), thus resulting in modification of HDL particles (50, 51). This mechanism may underlay the negative correlation between HDL-C and ApoAI levels with IL-6 concentration observed in our study. An additional mechanism of cytokine-induced hypocholesterolaemia includes a decrease in the hepatic synthesis and secretion of apolipoproteins (48, 52). This is in agreement with the negative correlation between CRP and ApoAI levels in this study. What is more, the negative correlation between changes in CETP activity and HDL-C levels during treatment suggests that the increased CETP activity during acute infection contributes to the reduction of HDL-C levels.

Different changes in total plasma Lp-PLA$_2$ activity, a marker of increased CVD risk (19, 53), have been observed in inflammatory diseases. Indeed, an increase in plasma Lp-PLA$_2$ activity in patients with sepsis (54, 55) or AIDS (21) has been reported. On the other hand, some studies showed that total plasma Lp-PLA$_2$ activity is decreased in sepsis (22, 56–58). In our study, total plasma Lp-PLA$_2$ and HDL-Lp-PLA$_2$ activities as well as the ratio of total plasma Lp-PLA$_2$/ApoB did not significantly differ between

patients on admission and controls (Table 2). Multiple regression analysis showed that, apart from LDL-C levels, the only significant predictor of plasma Lp-PLA_2 was TNFa concentration (Table 3). However, the correlation between Lp-PLA_2 and TNFa was negative (Table 3). Indeed, TNFa is considered as one potent inhibitor of Lp-PLA_2 secretion (59). On the other hand, total plasma Lp-PLA_2 activity may increase in response to Brucella-derived products, such as lipopolysaccharide (LPS) (60). LPS increases Lp-PLA_2 mRNA expression in several macrophage-rich tissues (60). Moreover, a previous study indicated the presence of anti-PAF antibodies in patients with Brucella (61). These antibodies may be the result of a specific anti-PAF response. PAF stimulates the synthesis and secretion of Lp-PLA_2, indicating a feedback regulatory mechanism that may serve to protect the host from the pathological effect of PAF (20). Thus, LPS and PAF or PAF-like substances may stimulate Lp-PLA_2 production and counteract the possible TNFa-mediated suppression.

One relevant question is where Lp-PLA_2 could be distributed in the presence of decreased LDL-C during infection. It is known that Lp-PLA_2 shows a preferential distribution for sdLDL particles compared with large buoyant ones (41). In agreement, sdLDL-C concentration increased during acute phase, while large LDL-C levels decreased compared with controls in our study (Table 2). Of note, total plasma Lp-PLA_2 and HDL-Lp-PLA_2 activities as well as the ratio Lp-PLA_2/ApoB did not significantly change 4 months after successful treatment compared with baseline.

Previous data suggested that PON1 may protect lipoproteins against oxidative modification, perhaps by hydrolyzing phospholipid and cholesteryl-ester hydroperoxides (24). Serum PON1 activity has been shown to be significantly lower in hepatitis C (62), human immunodeficiency virus (63), and *Helicobacter pylori* (64) infected patients. In accordance, PON1 (as expressed by either paraoxonase or arylesterase activity) was reduced in patients with acute Brucellosis compared with controls (Table 2). During infection and inflammation, serum PON1 activity decreases as a result of changes in synthesis and/or secretion of HDL particles and ApoAI (48, 50–52). Moreover, PON1 activity may decrease in response to Brucella-derived products, such as LPS (60). Indeed, serum PON1 activity decreases following endotoxin (LPS) administration in Syrian hamsters (65). Additionally, TNFa and IL-1 treatment of HepG2 cells results in a decrease in PON1 mRNA levels (65). A previous study demonstrated that oxidized lipids, which are formed during LDL oxidation, result in the inactivation of PON1 (66). Similarly, it is likely that a possible increased oxidative stress induced by Brucella infection could have led to an increased binding of free radicals to PON1, thus resulting in a less active PON1. However, in our study, we did not observe any difference in oxLDL levels or the oxLDL/ApoB ratio between patients and controls or during treatment. Low PON1 seems to be a risk factor for CVD (67). Therefore, this decrease in PON1 activity during infection could represent another factor linking the acute phase response with increased atherogenesis (24). An increase in PON1 activity (both paraoxonase and arylesterase) was seen 4 months following successful treatment (Table 2). This increase parallels that of HDL-C in the study cohort (Table 1).

We observed a shift in LDL subclass distribution toward smaller particles in patients with acute infection compared with controls. Indeed, in patients with acute Brucellosis, sdLDL-C levels increased, whereas large LDL-C levels and LDL size decreased compared with controls. Multiple regression analysis showed that significant predictors of sdLDL-C levels and LDL size were male sex, TGs, and IL-6 levels (Table 4). Of interest, 4 months later, sdLDL-C levels and LDL size were not significantly different compared with those on admission, whereas large LDL-C levels significantly increased (Table 2). There are limited reports in the literature concerning the effect of infection on LDL subclasses. Advanced stages of human immunodeficiency virus infection are associated with an atherogenic lipid profile, including a high prevalence of sdLDL particles (32). Moreover, severe periodontitis is associated with an increase in sdLDL-C levels (2). But which could be the reasons for the increased sdLDL-C and consequently decreased LDL size during acute infection in the absence of increased TGs? A possible explanation could be the independent correlation of sdLDL-C with IL-6. IL-6 may influence key enzymes in the metabolism of sdLDL, such as CETP and hepatic lipase. The finding that IL-6 decreased but did not fully reach control levels may partially explain the persistence of increased sdLDL-C 4 months later. It should be noted, however, that no significant correlation between changes in CETP activity (ΔCETP) and sdLDL-C levels (ΔsdLDL-C) as well as between ΔCETP and changes in IL-6 levels (ΔIL-6) during treatment were found (data not shown).

Finally, we measured ApoCII and ApoCIII, which are critical determinants of the TG-containing lipoprotein metabolism (68). Specifically, ApoCII is an important activator of LPL, thus enhancing the hydrolysis of TG-rich lipoproteins in the circulation. On the contrary, ApoCIII is a powerful inhibitor of LPL activity. We found a significant decrease of ApoCIII levels at baseline compared with controls and a subsequent increase following treatment. Despite that serum triglycerides did not differ between patients and controls, we noticed a negative correlation between change in ApoCIII (ΔApoCIII) and change in TGs (ΔTG; rho = -0.47, $P = 0.04$).

Study limitations

The number of study participants is relatively small. However, to our knowledge, this is the first study on the effects of acute infection with Brucella on serum lipids, lipoproteins, and associated enzymes. We used a cross-over design to compare subjects with matched healthy controls. Despite the limitations of this design, a prospective study of lipid alterations in this setting is not possible. In addition, the time that subjects were reevaluated (4 months after successful treatment) may have been too short for lipid alterations to be restored. However, it is very interesting to find that despite subjects being in excellent health, they still had lipid modifications.

CONCLUSIONS

Brucella infection is associated with atherogenic changes of serum lipids, lipoproteins, and associated enzymes, which are not fully restored 4 months following successful treatment. Whether these changes contribute to a possible infection-induced acceleration of atherogenesis requires further studies.

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