

## FABP4 plasma levels are increased in familial combined hyperlipidemia

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## ABSTRACT

The lipid profile of familial combined hyperlipidemia (FCHL) shares some characteristics with atherogenic dyslipidemia seen in diabetes, metabolic syndrome and obesity. Adipocyte fatty acid-binding protein (FABP4) appears to be a determinant of atherogenic dyslipidemia. We examined relationships between FABP4 plasma concentrations, dyslipidemia and metabolic variables in patients with FCHL. We studied 273 unrelated FCHL patients and 118 control subjects. FABP4 was higher in FCHL than controls, with mean levels of 21.8 (10.1)  $\mu\text{g/l}$  and 19.2 (9.2)  $\mu\text{g/l}$ , respectively (adjusted  $P=0.012$ ). In FCHL FABP4 correlated to BMI, waist circumference, insulin levels and HOMA index (all  $P<0.05$ ), but not to lipid levels, whereas in obese patients FABP4 correlated to triglyceride levels ( $r=0.303$ ,  $P=0.014$ ) and very low density lipoprotein size ( $r=0.502$ ,  $P=0.001$ ), as determined by nuclear magnetic resonance. Associations of FABP4 with BMI and waist circumference, but not with insulin levels, persisted in this subgroup. In conclusion, plasma FABP4 does not influence the lipid phenotype of FCHL. In a small subgroup of obese FCHL, FABP4 levels were associated with triglyceride-rich lipoproteins independent of insulin resistance. These results support a hyperlipidemic mechanism of FCHL different from similar metabolic conditions where fat mass is strongly related to FABP4 and hypertriglyceridemia.

**Supplementary Key words** dyslipidemia; FABP4; FCHL; insulin resistance; obesity

Familial combined hyperlipidemia (FCHL) is a common dyslipidemia that carries a high risk of cardiovascular disease. Indeed, it is estimated that about one in five patients surviving a premature myocardial infarction is a FCHL bearer (1). FCHL is a complex genetic disorder clinically characterized by variable phenotypic expression in both index cases and family members. Although hereditary transmission follows a dominant autosomic pattern, a unique gene or set of genes has not been identified for FCHL. Metabolic studies suggest that the main defect in FCHL is an increased production rate of triglyceride-rich lipoproteins, mainly liver-derived very low-density lipoproteins (VLDL) (2), with ensuing hyperlipidemia characterized by plasma increases of both triglycerides and cholesterol.

Hypertriglyceridemia is usually associated with the production of atherogenic small and dense low-density lipoproteins (LDL) and a tendency toward reduced high-density lipoprotein (HDL) cholesterol levels. Altogether, these metabolic derangements promote an increase in the number of circulating apolipoprotein B (apo B)-containing particles, which is reflected by an increased apo B plasma levels (3). This lipid profile, referred to as atherogenic dyslipidemia, is similar to that observed in patients with insulin resistance syndromes, such as diabetes, obesity, metabolic syndrome, and primary and secondary lipodystrophies (4). Because of this apparently common lipid phenotype, FCHL is considered to share a pathogenic basis with the above-mentioned diseases. Additionally, many FCHL patients fulfill the criteria for metabolic syndrome because they already have the necessary lipid defects. Several studies have implicated metabolic alterations of adipose tissue in the pathogenesis of FCHL (5-8). Among these studies, it has been reported that preadipocytes from patients with FCHL have upregulated CD36/FAT, which has been associated with an insulin resistance state (6). On the other hand, low levels of circulating adiponectin have been observed in patients with FCHL (7). Furthermore, differential expression of genes from subcutaneous adipose tissue in FCHL indicates overexpression of genes associated with inflammation and insulin resistance, such as tumor necrosis factor alpha, interleukin-6 and intercellular adhesion molecule-1. These data suggest an involvement of adipose tissue in the FCHL clinical phenotype (8).

The adipocyte fatty acid-binding protein, also known as FABP4, is highly expressed in adipose tissue, especially during adipocyte differentiation (9). FABP4 has been considered as a major cytoplasmic protein related to glucose and lipid metabolic functions (10,11). FABP4-deficient mice are protected from insulin resistance, hyperglycemia and atherosclerosis (12,13). Recently, it has been demonstrated that FABP4 is released into the human blood stream (14). The circulating FABP4 level has been associated with central adiposity, insulin resistance, and subclinical atherosclerosis (12,14,15). FABP4 has been postulated to be an early marker of metabolic syndrome and the future development of type 2 diabetes (16,17). In our previous studies, FABP4 plasma levels have been associated with metabolic syndrome in diabetic patients (9) as well as with the presence of metabolic syndrome and lipodystrophy in HIV-infected patients (18). We have also reported that FABP4 is a strong determinant of atherogenic dyslipidemia in diabetes (19). Although the mechanisms involved in this effect are unknown, they are probably linked to the hyperlipolytic state of these dysmetabolic conditions. Therefore, because FABP4 is currently considered a primary marker of adipose tissue metabolism derangement influencing the lipid profile, we hypothesized that if adipose tissue alterations have an important role in the clinical expression of FCHL, then FABP4 plasma levels will be associated with dyslipemia in FCHL. In this study, we determined for the first time FABP4 plasma levels in a large cohort of FCHL patients and related them to metabolic alterations.

## METHODS

### Study participants

Two hundred seventy-three patients with FCHL were included in the study. The criteria for FCHL diagnosis was based on the presence of primary combined hyperlipidemia, with off treatment serum LDL cholesterol (or total cholesterol if triglycerides > 3.38 mmol/l) > 90<sup>th</sup> percentile of the reference population or > 4.90 mmol/l (non-HDL cholesterol > 5.67 mmol/l if triglycerides > 3.38 mmol/l); triglycerides > 90<sup>th</sup> percentile of reference values or > 2.25 mmol/l; and at least one first-degree relative with hyperlipidemia (total cholesterol and/or triglycerides >90<sup>th</sup> percentile) or a first-degree relative with premature coronary heart disease. Participants were recruited from four lipid clinics in Spain. Patients with unstable angina, myocardial infarction within the last 3 months, daily alcohol intake >30 g, a weight change >10% in the last 3 months, or those participating in intensive physical training or a weight loss program were excluded a priori from entering the study. Demographic and anthropometric data and prior history of cardiovascular disease were recorded. A full physical examination was performed and fasting blood samples were collected. Any lipid lowering treatment was discontinued at least 4 weeks (6 weeks in the case of fibrates) before blood extraction. For purposes of comparing FABP4 levels in FCHL and in healthy subjects, we randomly selected a control group of 118 healthy subjects among those matched for age, gender and body mass index (BMI) from a general population database and plasma library (BIOBANC, Reus) belonging to the same geographical area. In order to assess the impact of adiposity on the FCHL phenotype, patients were subdivided by obesity status (BMI <30 kg/m<sup>2</sup> or ≥30 kg/m<sup>2</sup>) for further analyses. Metabolic syndrome in FCHL patients was defined according to National Cholesterol Education Program Adult Treatment Panel III (ATP-III) criteria (20). FCHL patients were categorized as having metabolic syndrome when they presented at least 3 of the following features: (a) central obesity (waist circumference ≥ 88 cm in women or ≥ 102 cm in men), (b) fasting glucose level ≥ 5.6 mmol/l or treatment with oral hypoglycemic agents, (c) hypertriglyceridemia (fasting triglycerides ≥

1.69 mmol/l), (d) low HDL-cholesterol (< 1.29 mmol/l in women or < 1.03 mmol/l in men), and (e) hypertension (blood pressure  $\geq$  130/85 mm Hg or antihypertensive treatment).

### **Biochemical analyses**

The basic biochemical parameters, including the standard lipid profile and glucose, insulin, apo B, and high-sensitivity C-reactive protein (hsCRP) were measured locally in fasting sera using standard enzymatic, immunoturbidimetric, chemiluminescent, RIA and immunonephelometric assays, respectively. The precision of these techniques, as described by coefficients of variation (CV) were <5%, <5%, <8%, and <8% interassay, respectively. Non-HDL cholesterol was calculated and used for clinical purposes and for the analyses included in this study instead of LDL-cholesterol because, by definition, the majority of FCHL patients had hypertriglyceridemia (19% with triglyceride levels > 4.5 mmol/l), which precludes the estimation of LDL-cholesterol by the Friedewald equation. Insulin resistance (IR) was estimated by using the homeostasis model assessment index (HOMA-IR), calculated as fasting glucose (in mmol/l) times fasting insulin (in mIU/l) divided by 22.5 (21). IR definition was established to be within the 75<sup>th</sup> percentile of our population (HOMA-IR  $\geq$  3.2). Plasma samples sent from each center to the Reus laboratory were used for measurement of FABP4 levels by a commercial ELISA kit (BioVendor Laboratory Medicine Inc., Brno, Czech Republic). The precision of this assay was 5.3% CV intra-assay and 3.9% CV interassay. The antibodies used in the human FABP4 ELISA are highly specific for human FABP4, with no detectable cross-reactivity to human FABP1, FABP2, FABP3 or FABP5. Results are expressed in "Système International" (SI) units.

### **Nuclear magnetic resonance lipid profile**

Plasma samples from the first 167 FCHL patients included in the study were used for the nuclear magnetic resonance (NMR) lipid profile study. Detailed lipoprotein subclassification (type, concentration and size) was performed on plasma using NMR spectroscopy (NMR LipoProfile; Raleigh, NC, USA) (22). Data are presented as molar particle concentrations

and sizes are given in nanometers. NMR data resulted in the following spectra: 3 subclasses of VLDL, including large VLDL and chylomicrons, medium VLDL, and small VLDL; intermediate-density lipoprotein (IDL); 4 subclasses of LDL, including large LDL, medium LDL, small LDL and very small LDL; and 3 subclasses of HDL, including large HDL, medium HDL and small HDL.

### Statistical analyses

Analyses were performed using SPSS (version 15.0, SPSS Inc., Chicago, IL, USA). All data are presented as means (SD) except when otherwise stated. Normality distribution was assessed with the Kolmogorov-Smirnov test. Log-transformation was performed before analyses when variables had a skewed distribution. The FCHL samples were divided into 2 subgroups of non-obese and obese patients according to BMI of  $<30$  or  $\geq 30$  kg/m<sup>2</sup>. One-way ANOVA and Fisher test were used for comparisons between 2 groups for continuous variables and for categorical variables, respectively. Univariate linear general models were used to adjust FABP4 values for waist circumference and multiple testing was corrected with Bonferroni testing by multiplying the univariate *P* value by the number of comparisons. Spearman correlation coefficients between FABP4 and other continuous variables were determined using a bivariate correlation test. FABP4 concentrations were categorized into sex-adjusted tertiles. A multiple linear regression analysis, including age, gender, waist circumference, insulin, cholesterol, and FABP4 plasma levels was performed to find the variables with an independent association with triglycerides in the obese FCHL subgroup. A binary logistic regression analysis was used to calculate the odds ratio (OR) for the association of raised FABP4 plasma concentrations (third tertile vs. second or first tertile) with the presence of metabolic syndrome. FABP4 first tertile was the reference group. The model included age, IR, hsCRP, apo B, LDL-cholesterol and FABP4 in sex-adjusted tertiles. Metabolic syndrome components were not included. *P* values shown, associated with the ORs, were obtained from a likelihood ratio test. A *P* value  $<0.05$  was considered statistically.

### **Statement of ethics**

We certify that all applicable institutional and governmental regulations concerning the ethical use of human volunteers were followed during this research. All subjects gave written informed consent, and each hospital's ethics committee approved the protocol design and the use of previously recorded databases and stored plasma samples for this study when necessary.

## RESULTS

The clinical characteristics of study groups are summarized in **Table 1**. FABP4 plasma levels in FCHL were significantly higher than in controls (**Table 1**). Women had significantly higher FABP4 levels than men, both in the FCHL group [25.5 (9.5)  $\mu\text{g/l}$  versus 20.0 (9.9)  $\mu\text{g/l}$ , respectively;  $P<0.001$  after Bonferroni correction] and in the control group [25.7 (9.3)  $\mu\text{g/l}$  versus 15.1 (6.4)  $\mu\text{g/l}$ , respectively;  $P<0.001$  after Bonferroni correction]. When subdivided by gender, FCHL men had higher FABP4 levels than controls [20.0 (9.9)  $\mu\text{g/l}$  versus 15.1 (6.4)  $\mu\text{g/l}$ , respectively;  $P<0.001$  after Bonferroni correction], while FCHL and control women had similar levels [25.5 (9.5)  $\mu\text{g/l}$  versus 25.7 (9.3)  $\mu\text{g/l}$ , respectively;  $P=0.947$ ].

Even though the control subjects were matched for BMI with the FCHL patients, the waist circumference of the FCHL group was significantly higher than that of the control group (**Table 1**). This was due to differences in the waist circumference of men [98.1 (8.0) cm in FCHL and 95.0 (10.6) cm in controls,  $P=0.028$  after Bonferroni correction]. Nevertheless, the differences in FABP4 observed between FCHL men and control men persisted after adjustment for waist circumference ( $P<0.001$  after Bonferroni correction).

Table 1 also shows basic clinical and biochemical characteristics of the FCHL population distributed according to the presence of obesity. FABP4 was higher in the obese group of FCHL patients compared to non-obese FCHL (**Table 1**).

In FCHL patients, FABP4 plasma levels were positively correlated with BMI, waist circumference, insulin levels and HOMA index (**Table 2**). No significant correlations existed between FABP4 and serum lipids in the overall FCHL group, but FABP4 levels were significantly correlated with triglyceride levels in the obese FCHL subgroup. The results of multiple linear regression analysis showed that FABP4 accounted for 17% of the variation in triglyceride levels in the obese FCHL subgroup ( $P=0.008$ ), independently of insulin levels. In the obese subgroup, FABP4 correlated to BMI and waist circumference, but not to insulin or the HOMA index (**Table 2**).

**Table 3** shows the correlations between FABP4 and the NMR lipid profile in all FCHL patients for whom NMR data were available and also for obese FCHL patients. No correlations were observed between FABP4 and any of the NMR lipid fractions when considering all the FCHL patients. However, FABP4 levels significantly correlated to large VLDL and chylomicron concentrations and VLDL size in the obese subgroup (**Table 3**).

Among the 273 subjects included in the study, 121 subjects (44%) fulfilled the ATP-III criteria for metabolic syndrome. FABP4 plasma levels were significantly higher in subjects with than in those without metabolic syndrome [23.9 (9.3)  $\mu\text{g/l}$  versus 20.4 (10.5)  $\mu\text{g/l}$ , respectively;  $P=0.001$  after Bonferroni correction]. By logistic regression analysis, FABP4 tertiles adjusted for gender, age, hsCRP, apo B, LDL-cholesterol and insulin resistance, were independently associated with the presence of metabolic syndrome ( $P<0.05$ ). Subjects with FABP4 plasma concentrations in the highest tertile ( $\geq 21.6$   $\mu\text{g/l}$  for men and  $\geq 28.0$   $\mu\text{g/l}$  for women) had a significantly increased likelihood of having the metabolic syndrome compared with those in the lowest tertile (OR 2.91; 95% CI, 1.32 to 6.41;  $P=0.007$ ).

## DISCUSSION

The main finding of our work is that FABP4 plasma levels are higher in FCHL patients than in controls. FABP4 was associated to obesity, metabolic syndrome, and insulin resistance markers. The FABP4 levels had no clear impact on the lipid profile when the whole FCHL population was considered, but were associated with hypertriglyceridemia in the presence of obesity. These results have interesting implications for the pathogenic mechanisms of dyslipidemia in FCHL. This primary alteration of lipid metabolism has been linked to other conditions showing a similarly disturbed lipid profile, including obesity, diabetes, and metabolic syndrome (4). In fact, there is a large body of evidence suggesting that alterations in adipose tissue play a crucial role in all of these conditions (23). FABP4 is considered a good plasma marker of adipose tissue dysfunction in these metabolic situations, being associated with central adiposity, metabolic syndrome, and insulin resistance (9,14,16). FABP4 plasma levels are clearly high in obesity, type 2 diabetes and metabolic syndrome (9,14,16), and we have previously reported that they are associated with triglyceride-rich lipoprotein components leading to atherogenic dyslipidemia (19). The lack of association between FABP4 and the lipid abnormalities of FCHL suggests that the pathogenic and metabolic bases of atherogenic dyslipidemia seen in these metabolic diseases are different. In FCHL various molecular defects likely contribute to similar clinical phenotypes. The abnormal lipid phenotype of FCHL has been attributed to defects in lipoprotein lipase (24) the APOA1-C3-A4-A5 gene cluster (25) or USF-1 (26) among others.

FCHL has also been reported to be associated with an increased prevalence of insulin resistance, as measured by the minimal model (27). In our study, the presence of insulin resistance did not influence the impact of FABP4 on the hyperlipidemia of FCHL. The clinical characteristic that most influenced the associations of FABP4 with FCHL was obesity. Obese FCHL patients had higher FABP4 levels, which contributed to higher triglycerides and to larger circulating VLDL leading to a more abnormal lipid profile. In fact, obese FCHL patients have a dual mechanism accounting for hyperlipidemia, including the still unknown genetic defect plus the acquired effects of adiposity. Interestingly, the association between

FABP4 and triglycerides in the obese FCHL subgroup was independent of insulin resistance markers. Similar results have been reported in obesity, diabetes and the metabolic syndrome (9,19), suggesting that FABP4 levels are markers of an adipocytic derangement paralleled by, but not dependent on, insulin resistance. That FABP4 levels are associated with large VLDLs in obese FCHL patients is also interesting. Lipoprotein kinetic studies have shown that VLDLs are secreted in two differentiated pools, large and small VLDL, according to the amount of fat they carry. In type 2 diabetic patients, greater visceral adipose tissue is associated with larger VLDL particles and higher VLDL particle number independently of BMI (28), and this lipoprotein pattern has been associated with an increased risk for atherosclerosis and cardiovascular disease (29). In this respect, the presence of obesity in FCHL patients could play a detrimental role for their lipid profile. Multifactorial analysis showed that 17% of the variation in triglyceride levels in the obese group could be attributed to FABP4. Another consideration derived from our results is the definition of metabolic syndrome in these patients. By definition, many FCHL patients fulfill the lipid criteria; therefore, the additional presence of hypertension, high blood glucose levels or obesity permits the clinical diagnosis of metabolic syndrome. However, the pathogenesis behind this cluster of risk factors is quite different in FCHL than in, for example, obese patients. Considering that FCHL is a cardiovascular risk factor *per se* and that its metabolic basis is different from that of other conditions associated with metabolic syndrome, the diagnosis of metabolic syndrome in the presence of this genetic lipid alteration should be restricted. Our data suggest that elevated FABP4 plasma levels could aid in the identification of the metabolic abnormalities associated with FCHL that lead to metabolic syndrome.

FABP4 was associated with hypertriglyceridemia only in the presence of obesity, defined both by BMI and waist circumference and independently of insulin resistance markers. However, because of the small number of obese patients in this group, these results need to be verified in larger cohorts. We have previously described a similar finding in diabetic patients (19), suggesting that FABP4 elevation is a metabolic defect paralleled to, but not dependent on, insulin resistance. In conclusion, the dyslipidemia seen in FCHL, although

sharing many characteristics with obesity, diabetes and the metabolic syndrome, is not associated with the adipose tissue alterations of which plasma levels of FABP4 are a marker. Although these data need to be replicated in larger populations, our data suggest that the presence of obesity in FCHL patients results in additional metabolic derangements leading to a greater triglyceride increase and larger VLDL signaled by increased plasma FABP4 levels.

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DISCLOSURES

None

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Table 1. Clinical characteristics of study groups

	Control group (n=118)	All FCHL patients (n=273)	<i>P</i> **	Non-obese FCHL patients (n=208)	Obese FCHL patients (n=65)	<i>P</i> **
Women, %	38	32	ns	33	29	ns
Age, years	48 (14)	47 (11)	ns	48 (11)	47 (10)	ns
BMI, kg/m <sup>2</sup>	27.4 (3.6)	27.8 (3.4)	ns	26.3 (2.3)	32.4 (2.1)	<0.001
Waist circumference, cm	93.2 (10.8)	96.0 (9.9)	0.024	93.0 (8.8)	105.2 (6.8)	<0.001
Glucose, mmol/l	5.0 (0.9)	5.5 (1.0)	<0.001	5.4 (0.8)	5.9 (1.4)	0.001
Insulin,* pmol/l	57.3 (40.5)	74.8 (61.5)	0.014	70.8 (59.5)	87.5 (66.2)	0.037
HOMA index *	1.8 (1.6)	2.4 (1.9)	0.001	2.2 (1.9)	2.9 (2.0)	0.005
hsCRP,* mg/l	-	3.4 (4.4)	-	2.9 (3.5)	5.0 (6.4)	<0.001
Lipoprotein B, mg/dl	105 (24)	153 (32)	<0.001	154 (31)	152 (33)	ns
Triglycerides,* mmol/l	1.32 (0.69)	3.27 (3.06)	<0.001	3.33 (3.31)	3.07 (2.09)	ns
Total cholesterol, mmol/l	5.40 (1.00)	7.42 (1.36)	<0.001	7.49 (1.41)	7.21 (1.14)	ns
LDL-cholesterol, mmol/l	1.46 (0.37)	1.13 (0.29)	<0.001	1.13 (0.28)	1.12 (0.31)	ns
LDL/HDL-cholesterol, mmol/l	3.95 (1.03)	6.30 (1.31)	<0.001	6.36 (1.37)	6.09 (1.09)	ns
FABP4,* µg/l	19.2 (9.2)	21.8 (10.1)	0.011	21.2 (10.5)	23.7 (8.6)	0.031

Values are means (SD) or frequencies.

BMI, body mass index; hsCRP, high-sensitivity C-reactive protein; HDL, high-density lipoprotein; FABP4, fatty acid-binding protein 4.

\* Log-transformed before analysis.

\*\**P* values corrected for multiple testing by Bonferroni correction.

Table 2. Correlations of plasma FABP4 levels in patients with FCHL.

	Control group (n=118)	All FCHL patients (n=273)	Obese FCHL patients (n=65)
Variable	r	r	r
Age, years	0.451 <sup>c</sup>	0.110	0.124
BMI, kg/m <sup>2</sup>	0.489 <sup>c</sup>	0.269 <sup>c</sup>	0.250 <sup>a</sup>
Waist circumference, cm	0.252 <sup>b</sup>	0.241 <sup>c</sup>	0.254 <sup>a</sup>
Glucose, mmol/l	0.202 <sup>a</sup>	0.055	0.142
Insulin, pmol/l	0.251	0.153 <sup>a</sup>	0.130
HOMA index	0.284 <sup>b</sup>	0.172 <sup>b</sup>	0.193
hsCRP, mg/l	-	0.192	0.136 <sup>a</sup>
Apolipoprotein B, mg/dl	0.272 <sup>b</sup>	-0.012	0.118
Triglycerides, mmol/l	0.049	0.101	0.303 <sup>a</sup>
Total cholesterol, mmol/l	0.241 <sup>b</sup>	-0.010	0.013
HDL-cholesterol, mmol/l	0.106	-0.004	-0.001
NonHDL-cholesterol, mmol/l	0.199 <sup>a</sup>	-0.011	0.033

<sup>a</sup> $P < 0.05$ ; <sup>b</sup> $P < 0.01$ ; <sup>c</sup> $P < 0.001$ .

r, Spearman correlation coefficient; BMI, body mass index; hsCRP, high-sensitivity C-reactive protein; HDL, high-density lipoprotein.

Table 3. FABP4 correlations with the nuclear magnetic resonance lipid profile in a subgroup of FCHL patients

Variable		All FCHL patients (n=167)		Obese FCHL patients (n=35)	
		r	P	r	P
VLDL and chylomicron concentrations, nmol/l	VLDL and chylomicrons	0.012	ns	-0.182	ns
	Large VLDL and chylomicrons	0.022	ns	0.383	0.023
	Medium VLDL	0.000	ns	-0.191	ns
	Small VLDL	0.017	ns	-0.138	ns
LDL concentrations, nmol/l	Total LDL	-0.061	ns	0.088	ns
	IDL	0.003	ns	0.221	ns
	Large LDL	0.000	ns	0.007	ns
	Small LDL	-0.043	ns	0.064	ns
	Medium LDL	-0.045	ns	0.021	ns
HDL concentrations, $\mu$ mol/l	Very small LDL	-0.039	ns	0.074	ns
	Total HDL	-0.075	ns	-0.015	ns
	Large HDL	-0.046	ns	-0.140	ns
	Medium HDL	0.017	ns	-0.039	ns
Mean sizes, nm	Small HDL	-0.078	ns	0.120	ns
	VLDL	0.034	ns	0.522	0.001
	LDL	-0.004	ns	-0.057	ns
	HDL	0.004	ns	-0.279	ns

r, Spearman correlation coefficient; VLDL, very low-density lipoprotein; LDL, low-density lipoprotein; IDL, intermediate-density lipoprotein; HDL, high-density lipoprotein.