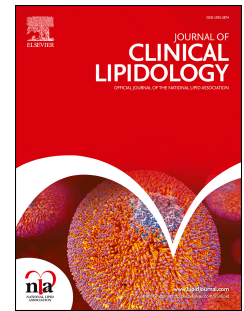


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Vascular inflammation and metabolic activity in hematopoietic organs and liver in familial combined hyperlipidemia and heterozygous familial hypercholesterolemia

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Vascular inflammation and metabolic activity in hematopoietic organs and liver in familial combined hyperlipidemia and heterozygous familial hypercholesterolemia

Short title: FDG uptake and familial dyslipidemias

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Abstract

Background: Familial dyslipidemias of either heterozygous (heFH) or combined (FCH) type lead to accelerated atherogenesis and increased cardiovascular risk.

Objective: The aim of this study is to investigate in statin-naïve adult patients with familial dyslipidemias whether inflammatory activation and liver, spleen and bone marrow metabolic activity differ compared to normolipidemic subjects and between dyslipidemic groups.

Methods: Fourteen patients with FCH, 14 with heFH and 14 normolipidemic individuals were enrolled. Serum lipids, high sensitivity C reactive protein (hs-CRP) and fibrinogen levels were measured, followed by ^{18}F FDG-PET/CT imaging. Radiotracer uptake in the aortic wall, spleen, bone marrow and liver was quantified as tissue to background ratio (TBR).

Results: Patients with heFH had significantly higher LDL levels compared to those with FCH and controls ($p<0.001$). However, aortic TBRs were higher in FCH compared to heFH patients and controls ($p=0.02$ and $p<0.001$ respectively). FCH patients exhibited higher FDG uptake in the spleen compared to controls ($p=0.05$). In addition, FCH exhibited higher bone marrow FDG uptake compared to heFH patients and controls ($p=0.03$ and $p=0.02$, respectively). FCH had higher liver uptake compared to heFH patients and controls ($p<0.001$ for both). Significant correlations were observed between inflammatory biomarkers and imaging indices as well as between aortic TBR and FDG uptake of hematopoietic organs and liver.

Conclusions: Systemic as well as vascular inflammation and spleen, bone marrow and hepatic metabolic activity are increased in patients with FCH, despite lower levels of LDL.

Key words: familial dyslipidemias, ^{18}F FDG pet/ct, vascular inflammation, spleen, bone marrow

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Introduction

Familial hypercholesterolemia is characterized by inflammatory activation demonstrated by elevated peripheral biomarkers in studies including mainly children and adolescents.¹⁻³ Furthermore, patients with familial hypercholesterolemia maintained an inflammatory phenotype despite long-term therapy with statins⁴. Recently, vascular inflammation has been investigated in a study of 24 patients with familial hypercholesterolemia and 14 normo-lipidemic controls, demonstrating that arterial wall inflammation was higher in patients with familial hypercholesterolemia under statin treatment in comparison with controls.⁵ Most of the existing studies do not distinguish between the two more common types of familial dyslipidemias: heterozygous familial hypercholesterolemia (heFH), which is associated with elevated LDL cholesterol levels and familial combined hyperlipidemia (FCH), which has been related with hypersecretion of very low density lipoproteins (VLDL), low HDL-cholesterol, small-dense LDL particles, and increased remnant cholesterol levels.^{6, 7} Moreover, recent evidence suggests that increased LDL cholesterol was correlated with atherosclerotic event risk but not with inflammation, whereas a causal association between remnant cholesterol and low-grade inflammation, as well as ischemic heart disease has been observed.⁸ It has also been shown that FCH influences hepatic metabolism⁹, insulin resistance and central obesity⁶ and increases hematopoietic tissue activation in the experimental setting.¹⁰

¹⁸F-fluorodeoxyglucose positron emission tomography/computed tomography imaging (¹⁸FDG-PET/CT) allows assessment of vascular inflammation¹¹⁻¹³ in tandem with hematopoietic tissue activation¹⁴⁻¹⁶, liver metabolic activity^{17, 18} and adipose tissue volumes¹⁹ and may therefore be an ideal imaging test for measuring those parameters in patients with FCH or heFH. No comparative assessment of the degree

of inflammatory activation in these two dyslipidemia types has been previously performed. Therefore, the aim of the present study was to investigate in statin-naïve adult patients with familial dyslipidemias whether inflammatory activation and liver metabolic activity differ compared to normolipidemic subjects and whether there are any differences between FCH and heFH patients.

Material and Methods

Study population

The study population was recruited from the Lipid Outpatient Clinic of First University Cardiology Clinic, Hippokration General Hospital, Athens, Greece from April 2015 to July 2016. Subjects with familial dyslipidemias were screened and we only included prospectively consecutive treatment-naïve patients with heFH and FCH. All participants were genetically unrelated. All subjects were informed about the protocol and after written informed consent were included in the study. All participants underwent ^{18}F FDG-PET/CT imaging. (Figure 1) Exclusion criteria for the dyslipidemic patients were known cardiovascular disease, active infections, inflammatory, autoimmune or active neoplastic disease (e.g. vasculitis, lymphomas), and uncontrolled diabetes mellitus.

The control group consisted of non-dyslipidemic individuals scheduled for ^{18}F FDG-PET/CT imaging. Subjects of the control group were individuals with a history of malignancy, free of active disease at the time of the ^{18}F FDG-PET/CT investigation. Patients with known atherosclerotic disease, presence of active systemic inflammatory or autoimmune disease or use of systemic corticosteroid treatment were excluded.

Data collection

Weight and height were measured for the calculation of body mass index (BMI) (kg/m^2). Blood lipid levels as well as serum high sensitivity C reactive protein (hs-CRP) and fibrinogen were measured in all the subjects after an overnight fasting. Total cholesterol, high-density lipoprotein cholesterol (HDL), and triglycerides were measured using colorimetric enzymic method in a Technicon automatic analyzer RA-1000 (Dade-Behring Marburg GmbH, Marburg, Germany). Serum insulin levels were determined by a solid-phase, competitive chemiluminescent enzyme immunoassay system, IMMULITE 2000. Apolipoprotein B and hs-CRP were measured by immunonephelometry (BN II system, Siemens GmbH, Germany and CRP reagent, Dade Behring, respectively). Fibrinogen was measured by standard coagulometry technique (Multifibren® U Reagent, Siemens). LDL cholesterol was calculated by the Friedewald formula, unless patients had triglycerides level >400 mg/dl. Insulin resistance was assessed by homeostasis model assessment (HOMA), by the use of the following equation: $\text{HOMA-IR} = (\text{glucose} \times \text{insulin})/405$ glucose in mg/dl, insulin in $\mu\text{U/ml}$, considering insulin resistance if HOMA was ≥ 3.20 . All biochemical analyses were carried out in one laboratory following the criteria of the World Health Organization Lipid Reference Laboratories.²⁰ The present study was approved by the Medical Research Ethics Committee of our Institution.

Diagnosis of heFH and FCH

Clinical diagnosis of heFH was based on criteria according to the US Make Early Diagnoses Prevent Early Deaths Program Diagnostic Criteria (MEDPED).²¹ Specifically, documentation of a LDL receptor gene defect, or off-treatment plasma LDL cholesterol level above the 95th percentile for sex and age, no secondary cause

of dyslipidaemia and at least one of the following: typical tendon xanthomas in the patient or in a first-degree relative, or familial vertical transmission, meeting LDL cholesterol criteria among first- or second-degree relatives, or proven coronary artery disease in the patient or in a first degree relative under the age of 60. In addition, Dutch Lipid Criteria score ≥ 3 was used in order to confirm the diagnosis.

The diagnosis of FCH was established when the patient and at least one family member had plasma triglyceride levels more than 133mg/dl and apolipoprotein B more than 120 mg/dl according to previously published criteria.²² Moreover, the presence of family history of premature cardiovascular disease was confirmatory of the diagnosis.

¹⁸F-FDG PET/CT imaging

¹⁸F-FDG PET/CT imaging was performed after a period of at least 6 hours fasting. Plasma glucose levels were <180 mg/dl. Images were obtained following intravenous administration of 140 μ Ci/kg FDG (Biocosmos S.A., Greece) and uptake period of 125.8 ± 14.8 min for patients with genetic dyslipidemias and 121.6 ± 6.6 min for the control group, respectively, $p=0.67$) ¹⁸F-FDG PET/CT (Biograph 6, Siemens, Forchheim) of the thorax and abdomen was performed with the patient placed in supine position. Attenuation-correction and co-registration was performed with the use of a low-dose, non-gated, non-contrast-enhanced computed tomography (30mA, 110 KV).

¹⁸F-FDG PET/CT measurements

¹⁸F-FDG PET/CT anonymized images were assessed by readers who were unaware of the patients' characteristics and the results of biochemical tests. In agreement with prior studies, ¹⁸F-FDG PET/CT images were assessed in consensus by two experienced physicians with an interest in cardiovascular nuclear medicine. Basic principles of image analysis have been previously described.²³ In brief, cross sections of circular or ellipsoid regions of interest (ROI) around the arterial wall were manually drawn along the whole vessel in consecutive axial images at intervals of 5 mm for the thoracic and abdominal aorta. For FDG uptake quantification, maximum standardized uptake value (SUVmax-standardized uptake value based in body weight) was recorded as the highest pixel activity within each ROI. To correct arterial PET signal for blood activity, 6 ROIs 3-4mm diameter at least, were placed over the superior vena cava and average SUVmean value was calculated. The arterial target-to-background ratio (TBR) was then derived by dividing the mean arterial SUVmax to the average value of venous SUVmean estimated from the superior vena cava (i.e: arterial TBR = mean arterial SUVmax /mean venous SUVmean).²³ TBR of thoracic aorta (TBR_{THORACIC}) was calculated as the average value of the TBRs of ascending aorta, aortic arch and descending aorta. Similarly, TBR of the abdominal aorta (TBR_{ABDOMINAL}) was calculated as the average value of suprarenal and infrarenal abdominal aorta. Furthermore, global TBR_{AORTA} was derived by calculating the sum of TBRs of ascending and descending aorta, aortic arch, suprarenal and infrarenal abdominal aorta divided by 5.

For the evaluation of the FDG uptake in the spleen, 3cm ROIs were designed on fused axial tomographic images from the display start to the end of the spleen image for each individual and SUVmax was determined. For performing comparisons between

groups and avoid weight based influences on our measurements, we have normalized spleen SUV values of each slice by calculating the ratio of SUVmax to the superior vena cava average, SUVmean. The splenic activity was then quantified as the absolute TBRmax from all the axial slices. Regarding bone marrow measurements, 5mm ROIs were placed within each vertebrae from the first thoracic to the fifth lumbar vertebrae and SUVmax was recorded. For consistency, with arterial and splenic measurements, TBRmax was then derived by dividing the SUVmax of each ROI with the blood pool average SUVmean. The metabolic activity of bone marrow was estimated as the average value of all the vertebrae TBRmax.¹⁶

¹⁸F-FDG PET/CT image analysis of the liver was performed by placing a circular ROI of 3 cm diameter in the right lobe in consecutive transverse images for each participant from the display start to the end of the liver image and SUVmax of each ROI was recorded.¹⁸ TBR liver was then calculated by the ratio of the absolute recorded hepatic SUVmax to the average value of SUVmean measured within the superior vena cava (SVC) as previously described (liverTBR = max liver SUVmax / mean venous SUV mean).

For correlations between imaging indices of different tissues and to avoid confounding factors such as weight (for SUV) or the influence of a common background (venous SUV mean, in the case of TBRs), we additionally calculated SUVmax normalized to the lean body mass (SUL) in spleen, bone marrow and liver.²⁴ SUL was calculated by the formula

$$\text{SUL} = \text{SUV} * (\text{LBM} / \text{PatientWeightGrams})^{24}$$

and LBM from

$$\text{LBM} = (9270 * \text{PatientWeightKg}) / (6680 + (216 * \text{BMI})) \text{ for men}$$

$$\text{LBM} = (9270 * \text{PatientWeightKg}) / (8780 + (244 * \text{BMI})) \text{ for women}^{25}$$

Finally, for measuring liver density by computed tomography (CT), 12 ROIs were drawn within axial CT images of the liver from three representative levels²⁶. Specifically, each axial image was divided in four sectors determined according to Couinaud segmentation system²⁶. Four ROIs were designed at each level with caution to avoid including large vessels and focal lesions. CT density was measured in Hounsfield units (HU), an index of hepatic fat distribution, as the average value derived from the 12 ROIs.^{17,27}

CT image analysis of Visceral Adipose Tissue

Image analysis of fused PET-CT images was performed using the Hermes Hybrid Viewer PDR 2.7.4 software (Hermes Medical Solutions, Stockholm, Sweden). First, a fat mask was created using the original CT images by selecting all voxels with attenuation between -190 and -30 Hounsfield Units (HU) (attenuation range corresponding to adipose tissue on CT). Then, the volume of interest (VOI) on CT was adjusted in order to include all visceral adipose tissue (VAT) voxels between the proximal (cephalic) end of the L1 and distal (caudal) end of the L3 vertebrae (subcutaneous adipose tissue was excluded from the VOI).

Statistical analysis

Quantitative data are presented as mean values \pm SD or medians (P25-P75), while qualitative variables as absolute and relative frequencies. Probability values are two-sided from the Mann-Whitney U test for continuous variables. When there were more

than 2 categories Kruskal-Wallis test was used. For between subgroups comparisons, Bonferroni correction was performed. Non-continuous values were compared by chi-square test. A value of $p < 0.05$ was considered significant. To assess correlations, Pearson's correlation coefficient was used. Statistical analysis was performed with commercially available software (version 20, SPSS Inc. Chicago, Illinois, USA).

Results

Study population

In total, 14 patients with heFH (7 with Dutch Lipid Criteria score 6, 6 with 8 and 1 with 9), 14 patients with FCH and 14 controls were included in the study. In table 1 are summarized the demographic, clinical and laboratory characteristics of the study population. FCH patients were mostly men, had more often arterial hypertension and were heavier. They exhibited higher VAT volume values compared to heFH patients and controls (2233.7 ± 776.5 versus 1120.8 ± 815.2 versus 806.3 ± 716.8 cm³, $p < 0.001$). VAT volumes of FCH patients were higher compared to heFH ($p = 0.001$) and controls (< 0.001).

ApoB levels were significantly higher in heFH compared to FCH group (193.14 ± 29.91 versus 142.29 ± 19.95 mg/dl respectively, $p < 0.001$). In contrast, FCH patients showed higher triglyceride and HOMA index values (326.50 ± 152.27 versus 110.86 ± 35.84 mg/dl, $p < 0.001$ and 3.25 ± 2.33 versus 1.56 ± 1.02 , $p = 0.04$, respectively).

Inflammatory biomarkers

FCH patients had significantly higher serum hs-CRP compared to heFH patients and controls (3.38 ± 3.03 versus 2.12 ± 2.85 versus 0.43 ± 0.24 mg/dl, respectively, $p=0.001$). Similar were the results for serum fibrinogen levels (364.00 ± 73.90 versus 301.42 ± 74.24 versus 262.19 ± 69.68 mg/dl, respectively, $p=0.008$). (Figure 2) Performing between groups comparisons FCH patients showed significantly higher hs-CRP and fibrinogen levels compared to controls ($p=0.009$ and $p=0.004$, respectively). In contrast the differences in inflammatory markers levels between heFH and FCH were not significant ($p=0.82$ and $p=0.24$, respectively). There were no differences between male and female patients in the levels of inflammatory markers within groups ($p=0.43$ and 0.7 for controls, 0.35 and 0.99 for heFH and 0.11 and 0.89 for FCH, for hs-CRP and fibrinogen respectively).

Vascular inflammation measurements

Patients with familial dyslipidemias showed higher aortic TBR values in thoracic, abdominal and global aorta compared to controls. (Figure 3) In particular, $TBR_{THORACIC}$ values were higher in FCH patients compared to heFH and controls (2.13 ± 0.22 vs. 1.95 ± 0.22 vs. 1.67 ± 0.10 , respectively, $p<0.001$). (Figure 3A) $TBR_{THORACIC}$ values of FCH patients were higher compared to controls ($p<0.001$). Similarly, $TBR_{THORACIC}$ values of heFH patients were higher compared to controls ($p=0.004$). There was a trend towards higher $TBR_{THORACIC}$ values of FCH compared to heFH patients ($p=0.06$).

$TBR_{ABDOMINAL}$ values were higher in FCH patients compared to heFH and controls (2.19 ± 0.32 versus 1.89 ± 0.21 versus 1.64 ± 0.25 , respectively, $p=0.001$, Figure 3B). $TBR_{ABDOMINAL}$ of FCH patients was higher compared to heFH ($p=0.02$) and controls

($p<0.001$). $TBR_{ABDOMINAL}$ values of heFH patients were higher compared to controls ($p=0.05$).

TBR_{AORTA} values were higher in FCH patients compared to heFH and controls (2.15 ± 0.24 versus 1.93 ± 0.20 versus 1.65 ± 0.14 , respectively, $p<0.001$, Figure 3C). TBR_{AORTA} values were higher in FCH patients compared to heFH ($p=0.02$) and controls ($p<0.001$). Similarly, TBR_{AORTA} values of heFH patients were higher compared to controls ($p=0.01$).

There were no gender related differences in vascular TBRs within the three study groups ($p=0.23$, 0.1 and 0.31 for controls, heFH and FCH, respectively). In addition, there was no difference in superior vena cava SUV_{mean} measurements between groups ($p=0.11$).

Correlation between lipid profile and arterial inflammation

There was a significant correlation between LDL levels and TBR_{AORTA} in heFH group but not in FCH group ($R=0.67$, $p=0.009$ and $R=-0.05$, $p=0.88$, respectively). No correlation was observed between triglycerides levels and TBR_{AORTA} . ($p=0.46$ for the FCH and $p=0.87$ for the heFH group, respectively). Similarly, no significant association between non-HDL levels and TBR_{AORTA} was found ($p=0.35$ for all the dyslipidemias and $p=0.17$ for the FCH group).

Hepatic metabolic activity

FCH patients exhibited higher TBR_{LIVER} values compared to heFH patients and controls (3.25 ± 0.46 versus 2.65 ± 0.33 versus 2.41 ± 0.27 , $p<0.001$, Figure 4A).

TBR_{LIVER} of FCH patients was higher compared to heFH ($p<0.001$) and to controls ($p<0.001$). In contrast, there was no difference in TBR_{LIVER} values between heFH patients and controls ($p=0.33$). No gender related differences in liver TBRs were observed within the three study groups ($p=0.41$, 0.2 and 0.82 for controls, heFH and FCH respectively). There was a significant correlation between TBR_{LIVER} and triglyceride levels in our study population ($R=0.6$, $p<0.001$).

FCH patients showed lower, although not statistically significant, hepatic CT density values compared to heFH and controls (54.52 ± 8.76 versus 59.92 ± 5.80 versus 60.98 ± 3.34 HU respectively, $p=0.06$).

Relationship of HOMA index with arterial and hepatic PET measurements

There was a strong correlation between HOMA levels and TBR_{AORTA} in patients with familial dyslipidemia ($R=0.57$, $p=0.007$). Similarly, HOMA values were significantly associated with hepatic FDG uptake ($R=0.47$, $p=0.038$).

Spleen and bone marrow metabolic activity measurements

There was a statistically significant difference in spleen TBR values between FCH, heFH patients and controls (2.65 ± 0.56 versus 2.22 ± 0.70 versus 2.02 ± 0.40 , $p=0.03$, Figure 4B). FCH patients exhibited higher TBR values compared to controls ($p=0.05$). However, there was no significant difference in spleen TBR values between FCH and heFH patients ($p=0.22$) or heFH and controls ($p=0.99$).

In addition, there was a statistically significant difference regarding bone marrow FDG uptake between FCH compared to heFH patients and controls (3.33 ± 0.55 versus

2.64±0.76 versus 2.50±0.65, $p=0.01$, Figure 4C). FCH patients exhibited higher TBR values compared to heFH patients ($p=0.03$) and controls ($p=0.02$). In contrast, there was no significant difference in bone marrow TBR values between heFH patients and controls ($p=0.99$).

There were no gender related differences in spleen and bone marrow TBRs within the three study groups ($p=0.56$, 0.73 and 0.81 for controls, heFH and FCH respectively for spleen and $p=0.62$, 0.99 and 0.78 for controls, heFH and FCH respectively for bone marrow).

Correlation of vascular inflammation with liver, spleen and bone marrow FDG uptake

Table 2 shows the correlation coefficients expressing the relationships between arterial TBRs with SULs of liver, spleen and bone marrow. Statistically significant correlations were observed for all three tissues.

Correlation of vascular, liver, spleen and bone marrow FDG uptake with inflammatory biomarkers

Table 3 illustrates the correlation coefficients expressing the relationships between vascular, liver, spleen and bone marrow FDG uptake values with hs-CRP and fibrinogen levels. Both circulating inflammatory biomarkers levels were associated with vascular, liver, spleen and bone marrow FDG uptake. No correlation was observed between hs-CRP or fibrinogen levels and mean venous SUVmean ($p=0.60$ and $p=0.39$, respectively). For demonstrative purposes, representative cases are illustrated in Figure 5.

Discussion

In the present study, we demonstrate that a) both systemic and local inflammation, as assessed by serum biomarkers and imaging methods respectively are increased in patients with familial dyslipidemias compared to non-dyslipidemic individuals, b) patients with FCH have higher arterial inflammation and metabolic activity of the major hematopoietic organs, despite lower LDL levels, compared to patients with heFH and controls.

Our results on increased low grade inflammatory activation as demonstrated by higher serum levels of hs-CRP and fibrinogen on dyslipidemic compared to normolipidemic individuals, are similar with previous observations¹, in which inflammatory cytokines were increased in children with heFH. Our findings are also in agreement with those of Cheng et al. and Real et al. who have shown that chronic inflammation as assessed by elevated CRP levels as well as other inflammatory biomarkers was present in adults patients with familial hypercholesterolemia^{3, 28}. Raised circulating lipids concentrations are probably implicated in the progression of atherosclerosis in familial dyslipidemias. In particular, atherogenic lipoproteins infiltrate into the arterial wall and they are oxidized in the subendothelial space driving inflammatory activation which consequently accelerate atherosclerosis.²⁹

FCH individuals seem to have a more intense inflammatory phenotype compared to those with heFH. Atherogenic dyslipidemia observed in the former comprises alterations of LDL composition, very low density lipoprotein (VLDL) overproduction and low HDL levels³⁰ triggering an intense inflammatory response. Enhanced inflammatory activity in FCH affects several organs leading to premature coronary

artery disease, hypertension and hepatic steatosis.⁶ We found that FCH patients showed increased vascular inflammation, as evaluated by arterial wall FDG uptake, compared to heFH and controls. Increased arterial TBR values were also observed in a previous study of patients with familial hypercholesterolemia, however, no comparison between individuals with different types of familial dyslipidemias regarding arterial inflammation was performed.⁵ In our study, vascular inflammation was more intense in FCH compared to heFH subjects, despite lower LDL levels in the FCH group. Moreover, a significant correlation between LDL levels and the degree of arterial inflammation was found only in heFH group. Three mechanisms may be contributing to these findings: Firstly, the small-dense LDL particles observed in FCH, and not in heFH patients, may explain the increased inflammatory atherosclerotic burden.^{6, 31} In particular, small dense LDL particles showed an enhanced atherogenicity probably due to higher infiltration within the arterial wall, prolonged half-life and reduced binding for the LDL-receptor making them more prone to oxidative alternation³¹⁻³³. Secondly, the insulin resistance previously observed in FCH³⁴, leads to release of free fatty acids from adipocytes and consequently, by blocking of glucose oxidization, to hepatic overproduction of triglycerides and apolipoprotein B rich lipoproteins³⁵ which have proinflammatory activation in the vasculature. In agreement with the study by Pihlajamaki³⁴ J et al, we also found increased insulin resistance in FCH patients. In addition, a strong correlation between HOMA index and vascular FDG uptake was present in the current study. A similar association between the same parameters has also been reported by Ai Haraguchi et al.³⁶ in the general population. Finally, adipose tissue metabolism dysfunction, consisted of reduced production of the atheroprotective adiponectin and increased atherogenic leptin³⁷ and reduced storage rates of

triglycerides in fat in FCH³⁸, contributes to accelerated atherosclerosis in patients with FCH.

In support of the aforementioned findings, we observed that patients with FCH showed elevated metabolic activity of the spleen and bone marrow compared to other groups. Recent evidence suggests that inflammatory activity in atherosclerosis is initiated in the bone marrow and spleen^{39, 40}. Other studies report that in response to hypercholesterolemia, the bone marrow and spleen overproduce Ly-6C high monocytes that enter the circulation, preferentially infiltrate in atherosclerotic lesions, and convert to macrophages^{40, 41}. In addition, two recent studies, using ¹⁸FDG-PET/CT measurements, state that remnant cholesterol induces enhanced proliferation at the bone marrow level and that a chronically affected hematopoietic system was the main driver for a low-grade inflammatory activity in atherosclerosis.^{14, 42} In light of these studies, the increased metabolic activity of haematopoietic tissue in patients with FCH may be a marker of increased immune response and related to macrophage accumulation in atherosclerotic lesions.

Liver FDG uptake in FCH patients was increased compared to heFH and non-dyslipidemics. The increase in FDG uptake by the liver may due to a number of factors associated with the FCH phenotype, such as insulin resistance, obesity, and hypersecretion of VLDL. Insulin resistance is common in FCH patients and is associated with lipid phenotype expression and fatty liver disease.⁴³ In the current study, HOMA index values were associated with liver FDG uptake and were higher in FCH compared to heFH patients. In accordance with previous studies, we observed a significant association between the biological activity of the spleen and bone marrow with arterial inflammation that was even stronger for the abdominal aorta.^{15, 16} These findings indicate that the inflammatory process during atherosclerosis is possibly

modified by monocytes and progenitor cells produced by the spleen and bone marrow. Moreover, in agreement with recent data⁴⁴, we also found an association between liver FDG uptake and arterial inflammation. Probably, liver fat accumulation and insulin resistance modify production of triglycerides-rich lipoproteins in the liver driving a pro-atherothrombotic lipid profile and atherosclerosis.⁴³

Clinical implications

The prognostic role of arterial, spleen and bone marrow FDG uptake for future cardiovascular events of asymptomatic individuals has been previously demonstrated.^{15, 19, 45} The present study contributes to our understanding of the underlying pathophysiology in hereditary dyslipidemias. There is evidence that patients with heFH are at an increased risk not only for premature but also for recurrent cardiovascular events⁴⁶. Considering the high prevalence of both heFH and FCH and the differences in vascular inflammation and immune system activation between the two entities, systematic screening of general population for hereditary dyslipidemias, as well as early diagnosis and appropriate treatment modification are of paramount importance for these patients. Finally, our demonstration of an intense atheromatic milieu in FCH patients supports the need for development of new therapeutic strategies addressing the drivers of risk and inflammation that is atherogenic dyslipidemia, insulin resistance and remnant lipoproteins. Such strategies can be targeted to patients for whom Mediterranean diet, exercise, control of obesity and visceral fat burden, as well as intensive medical management for controlling triglycerides and overall atherogenic lipoprotein burden (non-HDL-C and apoB levels) resulted in a sub-optimal risk reduction for future cardiovascular events.

Limitations

The present study has several limitations but also some strengths. Firstly, a modest number of patients with familial dyslipidemias were included. However, in our study only treatment-naïve adults with different types of hereditary dyslipidemias underwent ^{18}F -FDG PET/CT imaging, and these are unique features compared to prior similar studies. Moreover, we have employed ^{18}F -FDG PET/CT imaging that is highly reproducible and sensitive as it can detect FDG signal differences between groups with a relatively small number of patients.⁴⁷ Secondly, the FCH group comprised predominantly male individuals, however, we observed no gender differences in FDG uptake in patients with heFH or controls. Thirdly, FCH shares considerable phenotype overlap with metabolic syndrome. Indeed, FCH patients had significantly higher BMI than heFH and controls in our study. However, FCH patients were recruited according to validated criteria.²² Moreover, quantification of pericardial fat was not performed because the quality of CT images with the instrumentation used in the current study would not have allowed reliable measurements. Lastly, the contribution of inflammation in FDG signal differences in the other tissues was identified indirectly by the increased levels of inflammatory markers. We have not performed biopsies in these organs or flow cytometry analysis of peripheral monocytes, because in depth investigation of underlying pathophysiological mechanisms or causality was beyond the scope of this study.

Conclusions

Both systemic and local inflammation, are increased in patients with familial dyslipidemias compared to non-dyslipidemic individuals. In addition, compared to patients with heFH and controls, individuals with FCH have higher arterial inflammation and metabolic activity of the major hematopoietic organs and liver as expressed by FDG uptake, despite lower LDL levels. These differences could imply a higher risk of patients with FCH compared to those with heFH, and partially explain why individuals with familial dyslipidemias and similar risk factors and LDL levels do not develop atherosclerosis to the same extent.

Contributions

Konstantinos Toutouzas: conception and design of the study, analysis and interpretation of data and drafting of the manuscript.

John Skoumas: conception and design of the study and analysis and interpretation of data.

Iosif Koutagiar: design of the study, collection, analysis and interpretation of data.

Georgios Benetos: analysis and interpretation of data and drafting of the manuscript.

Nikoletta Pianou: analysis and interpretation of data and drafting of the manuscript.

Alexandros Georgakopoulos: analysis and interpretation of data and drafting of the manuscript.

Spyros Galanakos: analysis and interpretation of data and drafting of the manuscript.

Alexios Antonopoulos: analysis and interpretation of data and drafting of the manuscript.

Maria Drakopoulou: analysis and interpretation of data and drafting of the manuscript.

Evangelos K. Oikonomou: collection, analysis and interpretation of data, drafting of the manuscript

Pavlos Kafouris: analysis and interpretation of data and drafting of the manuscript.

Emmanouil Athanasiadis: analysis and interpretation of data and drafting of the manuscript.

Marinos Metaxas: collection and interpretation of data, drafting of the manuscript.

George Spyrou: collection and interpretation of data, drafting of the manuscript.

Zoi Pallantza: drafting of the manuscript and revising it critically for important intellectual content.

Nikolaos Galiatsatos: collection, analysis and interpretation of data, drafting of the manuscript

Constantina Aggeli: drafting of the manuscript and revising it critically for important intellectual content.

Charalampos Antoniadis: drafting of the manuscript and revising it critically for important intellectual content.

Georgia Keramida: drafting of the manuscript and revising it critically for important intellectual content.

Adrien M. Peters: drafting of the manuscript and revising it critically for important intellectual content.

Constantinos D. Anagnostopoulos: conception and design of the study, drafting and revising the manuscript critically for important intellectual content and final approval of the manuscript submitted.

Dimitris Tousoulis: revising the manuscript critically for important intellectual content and final approval of the manuscript submitted.

Disclosures

There is no conflict of interest to disclose for any author. All authors have approved the final article.

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Figure Legends

Figure 1: Flow chart of the study.

Figure 2: Comparison of inflammatory biomarkers levels in patients with familial combined hyperlipidemia (FCH), heterozygous familial hypercholesterolemia (heFH), and nondyslipidemics controls. Individuals with FCH exhibited higher high sensitivity- C reactive protein (hs-CRP) (A) and fibrinogen (B) values in comparison with heFH and controls. The bottom of the boxes represents the first quartile, the top of the boxes represents the third quartile, and the line in the boxes represents the median value.

Figure 3: Fluorodeoxyglycose signal quantified as target-to-background ratio (TBR) within the wall of the thoracic aorta (A), abdominal aorta (B), and whole aorta (C), is greater in patients with familial combined hyperlipidemia (FCH) compared to individuals with heterozygous familial hypercholesterolemia (heFH) and nondyslipidemic controls. The bottom of the boxes represents the first quartile, the top of the boxes represents the third quartile, and the line in the boxes represents the median value.

Figure 4: Fluorodeoxyglucose uptake within the liver (A), the spleen (B) and the bone marrow (C) of subjects with familial dyslipidemias and of nondyslipidemic individuals. The bottom of the boxes represents the first quartile, the top of the boxes represents the third quartile, and the line in the boxes represents the median value.

Figure 5: Increased hepatic, spleen, aortic and bone marrow fluorodeoxyglycose (FDG) uptake in patients with familial combined hyperlipidemia (FCH). Fused ^{18}F -FDG PET/CT axial views of liver (green crosses) and spleen (marked as white *) (A1, B1, C1), coronal views of

aorta (blue arrows) (A2, B2, C2), and sagittal views of bone marrow (red arrows) (A3, B3, C3). There was an intense FDG uptake quantified as target-to-background ratio (TBR) within liver, spleen, aortic wall and bone marrow in patients with FCH (A1-3) compared to subjects with heFH (B1-3) and controls (C1-3).

Table 1: Baseline characteristics and lipid profile of the study population

	Controls (n=14)	heFH (n=14)	FCH (n=14)	p
Age	42.00 (30.00- 55.50)	49.50 (32.25- 60.00)	44.50 (35.75- 52.00)	0.60
Gender (male)	6 (46.2)	6 (42.9)	13 (92.9)	0.01
Diabetes mellitus	2 (15.4)	1 (7.1)	3 (21.4)	0.56
Arterial Hypertension	6 (46.2)	2 (14.3)	7 (50)	0.10
Family history of CAD	0 (0)	9 (64.3)	5 (35.7)	0.002
Current smoker	9 (69.2)	5 (35.7)	8 (57.1)	0.21
BMI	21.74 (17.46- 23.79)	24.17 (22.28- 27.77)	30.65 (25.80- 34.75)	<0.001
Glucose (mg/dl)	100.00 (95.00- 122.00)	88.00 (83.75- 94.75)	99.00 (94.00- 111.25)	0.005
Total cholesterol (mg/dl)	165.00 (151.50- 175.50)	321.50 (300.50- 350.75)	264.00 (230.50- 306.00)	<0.001
LDL cholesterol (mg/dl)	97.00 (91.00- 103.00)	238.00 (209.75- 269.50)	162.00 (145.00- 184.00)	<0.001
HDL cholesterol (mg/dl)	43.00 (40.50- 45.50)	48.50 (44.75- 73.50)	36.50 (29.75- 44.75)	0.001
Triglycerides (mg/dl)	88.00 (79.00- 93.50)	98.50 (86.50- 128.00)	260.00 (232.00- 399.25)	<0.001

CAD indicates coronary artery disease; BMI, body mass index; LDL, low density lipoprotein; HDL, high density lipoprotein; All values are expressed as median (P25-P75) or n (%).

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Table 2: Correlation coefficients between liver, spleen, bone marrow SULs and vascular TBRs

	TBR _{THORACIC}	TBR _{ABDOMINAL}	TBR _{AORTA}
SUL _{LIVER}	0.36 (0.04)	0.47 (0.004)	0.40 (0.02)
SUL _{SPLEEN}	0.49 (0.006)	0.47 (0.005)	0.47 (0.007)
SUL _{BONEMARROW}	0.38 (0.03)	0.45 (0.005)	0.41 (0.02)

SUL indicates standardized uptake value normalized by lean body mass; TBR, target to background ratio. All values are expressed as correlation coefficients R (p values).

Table 3: Correlation coefficients of vascular, liver, spleen and bone marrow inflammation with inflammatory biomarkers

	TBR _{THORACIC}	TBR _{ABDOMINAL}	TBR _{AORTA}	TBR LIVER	TBR SPLEEN	TBR _{BONE} MARROW
hs-CRP	0.49 (0.02)	0.54 (0.006)	0.52 (0.01)	0.63 (0.001)	0.64 (0.001)	0.45 (0.03)
Fibrinogen	0.65 (<0.001)	0.51 (0.005)	0.64 (<0.001)	0.53 (0.004)	0.54 (0.004)	0.56 (0.02)

TBR indicates target-to-background ratio; hs-CRP, high sensitivity C reaction protein.

All values are expressed as correlation coefficients R (p values).

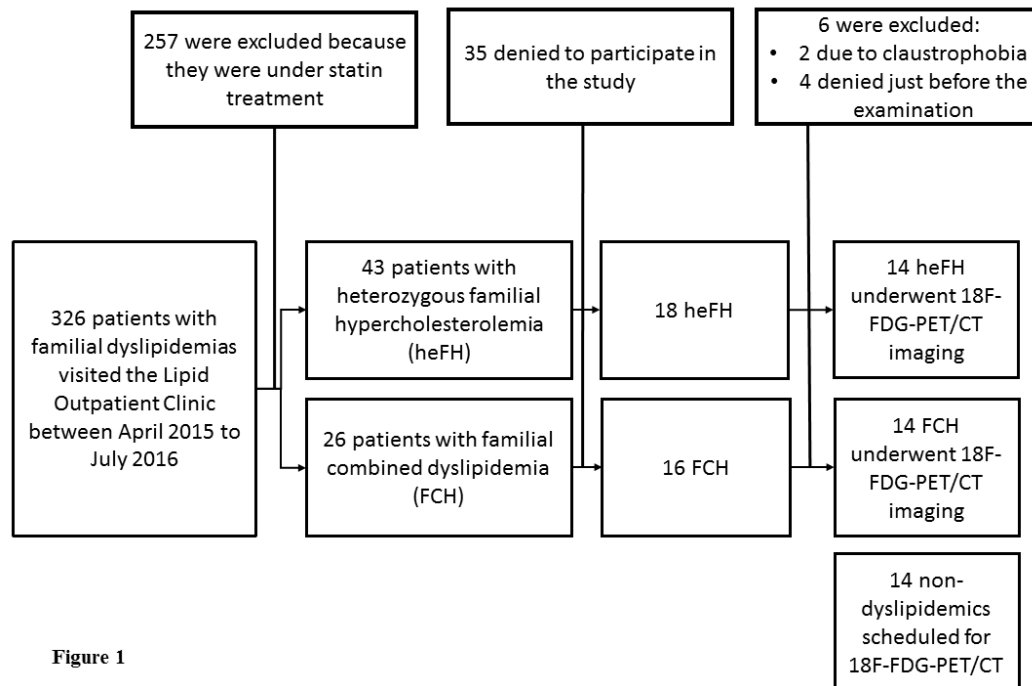


Figure 1

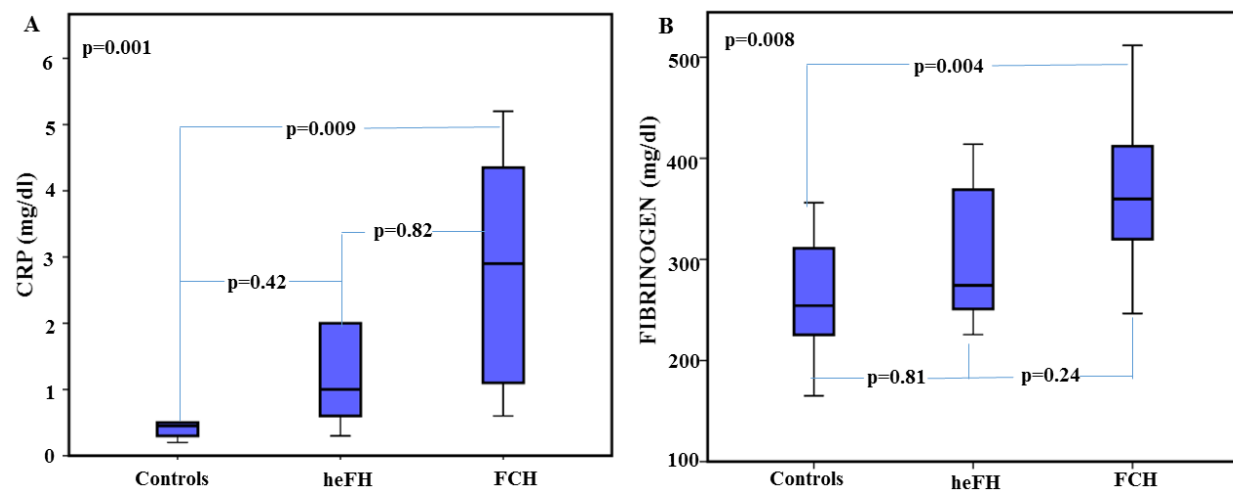


Figure 2

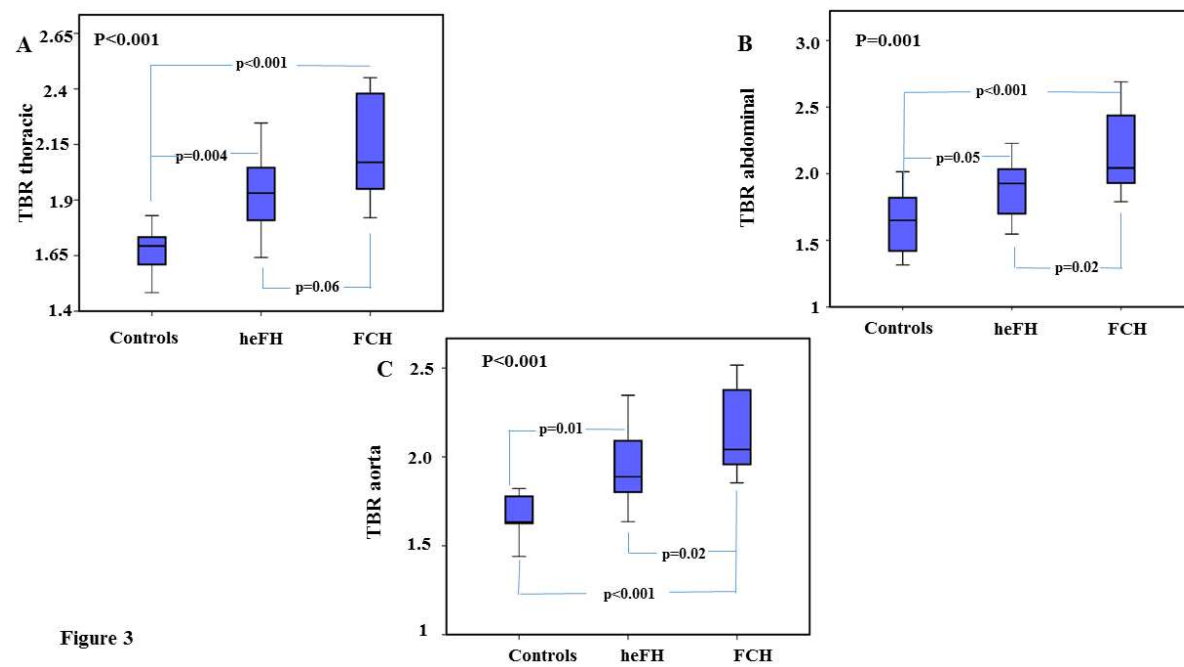


Figure 3

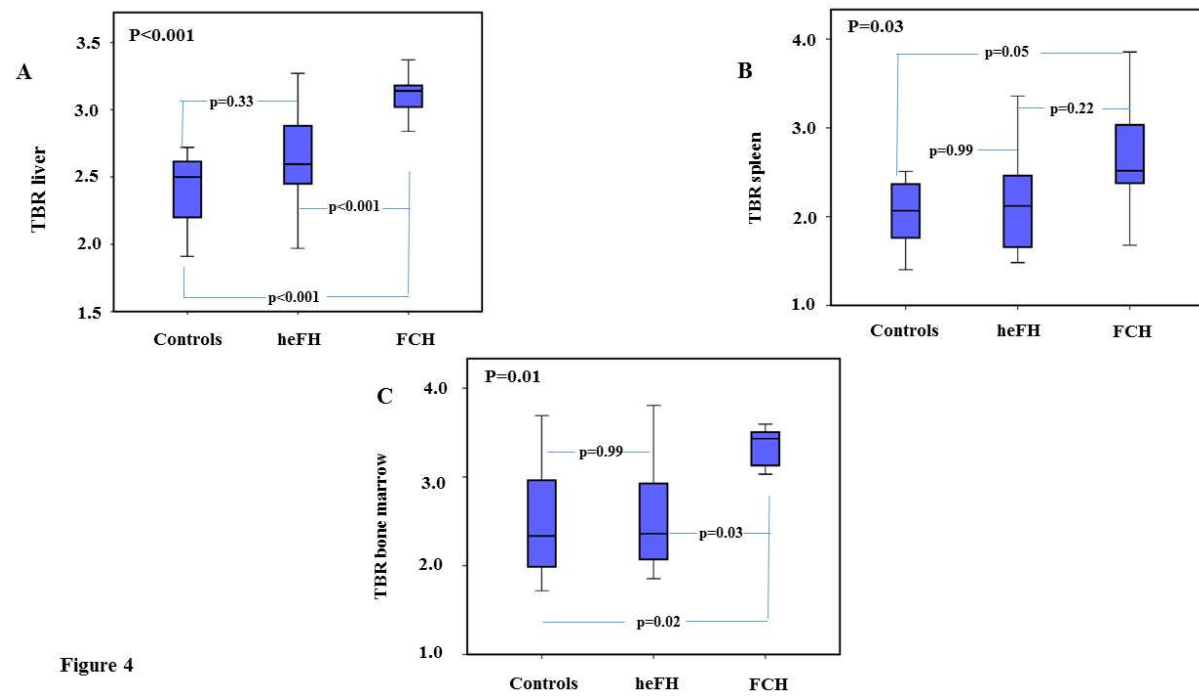


Figure 4

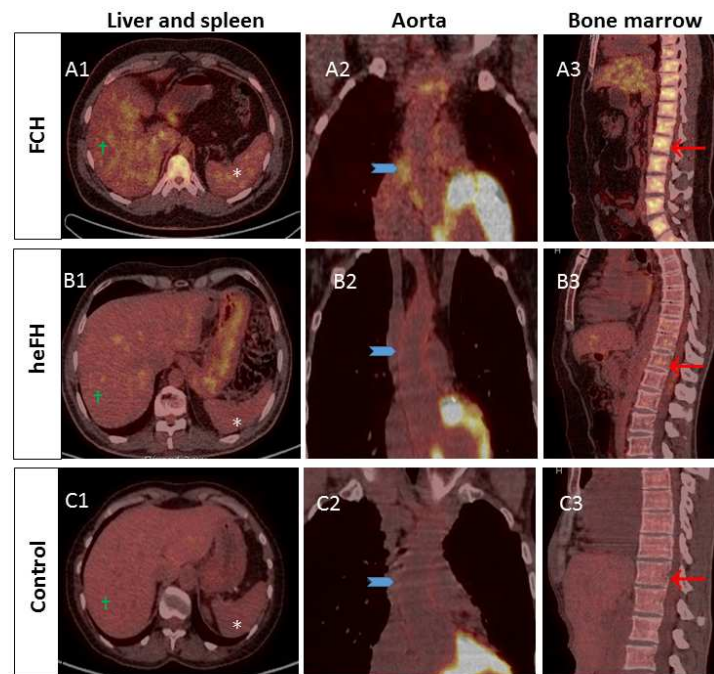


Figure 5

Highlights

- This is the first study to investigate vascular inflammation in familial combined dyslipidemia
- Liver, bone marrow and spleen metabolic activity were also evaluated
- FCH exhibits higher vascular activity compared to heFH
- Correlations between vascular and hematopoietic tissues metabolic activity were noted