Coronary Flow Reserve in Young Men With Familial Combined Hyperlipidemia

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Background—Familial combined hyperlipidemia (FCHL) is a common hereditary disorder of lipoprotein metabolism estimated to cause 10% to 20% of premature coronary heart disease. We investigated whether functional abnormalities exist in coronary reactivity in asymptomatic patients with FCHL.

Methods and Results—We studied 21 male FCHL patients (age, 34.8±5.4 years) and a matched group of 21 healthy control subjects. Myocardial blood flow (MBF) was measured at baseline and during dipyridamole-induced hyperemia with PET and 15O-labeled water. The baseline MBF was similar in patients and control subjects (0.79±0.19 versus 0.88±0.20 mL·g⁻¹·min⁻¹, P=NS). An increase in MBF was seen in both groups after dipyridamole infusion, but MBF at maximal vasodilation was lower in FCHL patients (3.54±1.59 versus 4.54±1.17 mL·g⁻¹·min⁻¹, P=0.025). The difference in coronary flow reserve (CFR) was not statistically significant (4.7±2.2 versus 5.3±1.6, P=NS, patients versus control subjects). Considerable variability in CFR values was detected within the FCHL group. Patients with phenotype IIB (n=8) had lower flow during hyperemia (2.5±1.2 versus 4.2±1.5 mL·g⁻¹·min⁻¹, P<0.05) and lower CFR (3.4±2.1 versus 5.4±2.0, P<0.05) compared with phenotype IIA (n=13).

Conclusions—Abnormalities in coronary flow regulation exist in young asymptomatic FCHL patients expressing phenotype IIB (characterized by abnormalities in both serum cholesterol and triglyceride concentrations). This is in line with previous observations suggesting that the metabolic abnormalities related to the pathophysiology of FCHL are associated with the phenotype IIB. (Circulation. 1999;99:1678-1684.)

Key Words: lipids ■ blood flow ■ myocardium ■ hyperlipidemia, familial combined ■ tomography

Familial combined hyperlipidemia (FCHL) is the most common inherited disorder of lipid metabolism. The prevalence of FCHL is 1% to 2% in the general population,1–4 and it is estimated to cause about 10 to 20% of the incidence of premature coronary heart disease (CHD).1,5–11 The affected subjects exhibit elevations of total cholesterol (phenotype IIA), triglycerides (TG) (phenotype IV), or both (phenotype IIB).1–4 Despite intensive research, the precise genetic or metabolic defects in FCHL have not yet been resolved. However, recent data from our group provided the first evidence of linkage to a subchromosomal region (1q21–23) in Finnish FCHL families, thus confirming that FCHL exists as a genetic entity.12 The linkage was strongest in families that included subjects with the lipid phenotype IIB. This is in line with observations suggesting that the key metabolic abnormalities related to the pathophysiology of FCHL are most strongly associated with the phenotype IIB.13 In our opinion, phenotype IIB is the most characteristic marker of FCHL to date, provided that strict age- and sex-specific criteria are used.14

PET can be used to measure regional myocardial blood flow accurately without invasive and potentially risky procedures.15–18 Measuring myocardial blood flow at rest and after dipyridamole or adenosine administration allows calculation of coronary flow reserve. Impaired coronary flow reserve has been suggested as a surrogate measure of subclinical coronary atherosclerosis, providing an integrating measure of vascular endothelial function and smooth muscle relaxation.19 We have shown that coronary flow reserve is impaired already in young patients with familial hypercholesterolemia (FH) and insulin-dependent diabetes mellitus (IDDM)20,21 and furthermore that it becomes impaired even in young healthy control subjects expressing moderately elevated conventional vascular risk factors22 or high levels of oxidized LDL.23

Because FCHL is associated with premature coronary heart disease and because heterogeneity appears to exist in the...
metabolic disturbances within FCHL, related to the lipid phenotype expression, the aim of the present study was to investigate whether there are abnormalities in the coronary flow reserve in young asymptomatic patients with FCHL and whether they are related to the lipid phenotype expression.

**Methods**

**Subjects**

Twenty-one men with FCHL and a mean age of 34.8 ± 6.6 years were enrolled in this study. Patients were selected from the register of European Multicenter Study on Familial Dyslipidemias (EUFAM study), the purpose of which is to obtain large, carefully documented European families with premature coronary heart disease. The general inclusion criteria in EUFAM study for the FCHL probands are (1) age of 30 to 55 years for men and 30 to 65 years for women; (2) ≥ 50% stenosis in ≥ 1 coronary artery; (3) serum total cholesterol, HDL cholesterol, and LDL cholesterol level outside the age- and sex-specific 90th percentile; (4) ≥ 5 accessible first-degree relatives; and (5) no signs of type I diabetes mellitus, thyroid disease, hypertension or current smoking. None of the subjects were on continuous medication. Thirteen of the patients were classified as phenotype IIA and 8 patients as phenotype IIB. No patients were found to have phenotype IV. A group of 21 healthy volunteers matched for age and blood pressure served as a control group. The subject characteristics are shown in Tables 1 and 2. The study protocol was accepted by the Joint Commission on Ethics of the Turku University and the Turku University Central Hospital. Each subject gave written informed consent.

**Study Design**

All PET studies were performed after a 6-hour fast. Alcohol and caffeine were prohibited 12 hours before the study. The subjects were lying supine in the PET scanner. Two catheters were inserted: 1 in the antecubital vein of the left hand for injection of [15O]H2O and dipyridamole, and 1 in the antecubital vein of the right hand for blood sampling. Myocardial perfusion was measured twice, once at rest and once after administration of dipyridamole. Heart rate and blood pressure were monitored during the studies to calculate the rate-pressure product. ECG was continuously monitored during the PET studies.

**Production of [15O]CO and [15O]H2O**

For production of [15O], a low-energy deuteron accelerator Cyclone 3 was used (Ion Beam Application Inc). [15O]CO was produced in a conventional way.24 [15O]-labeled water was produced with dialysis techniques in a continuously working water module.25 Sterility and pyrogenicity tests for water and chromatographic analysis for gases were performed to verify product purity.

**Image Acquisition, Processing, and Correction**

The patients were positioned supine in a 15-slice ECAT 931/08–12 tomograph (Siemens/CTI Inc) with a measured axial resolution of 6.7 mm and 6.5 mm in-plane. After a transmission scan, the subjects’ nostrils were closed, and they inhaled [15O]CO for 2 minutes through a 3-way inhalation flap valve (0.14% CO mixed with room air; mean dose, 3139±608 MBq [85±16 mCi]). After inhalation, carbon monoxide was allowed to combine with hemoglobin in red blood
cells for 2 minutes before a 4-minute static scan was started. During the scan period, 3 blood samples were drawn at 2-minute intervals, and blood radioactivity was measured immediately. A 10-minute period was allowed for $[^{15}\text{O}](\text{H}_2\text{O})$ radioactive decay before the flow measurements.

Flow was measured at baseline and 2 minutes after the end of intravenous administration of dipyridamole ($0.56\,\text{mg/kg}$ over 4 minutes). Then, $1696\,\text{Bq}$ at baseline and $1702\,\text{Bq}$ after dipyridamole, $P<0.05$, were injected intravenously for 2 minutes ($1667\,\text{s}$), and dynamic scanning was started for 6 minutes (6 times for 5 seconds, 6 times for 15 seconds, and 8 times for 30 seconds). All data were corrected for dead time, decay, and photon attenuation and reconstructed in a $128 \times 128$ matrix. The final in-plane resolution in reconstructed, and Hann-filtered (0.3 cycles per second) images were 9.5 mm full width at half maximum.

**Calculation of Regional Blood Flow**

Large regions of interest (ROIs) were placed on representative transaxial ventricular slices in each study covering the anterior and lateral free walls of the left ventricle. The ROIs were drawn on the images obtained at rest and copied to the images obtained after dipyridamole administration. Values of regional myocardial blood flow (expressed in $\text{mL} \cdot \text{g}^{-1} \cdot \text{min}^{-1}$) were calculated according to the previously published method using the single compartment model.26–27 Because no regional differences were found in myocardial perfusion and to enhance the accuracy of the measurements, the mean blood flow values at rest and after dipyridamole were calculated and used in further analyses. The arterial input function was obtained from the left ventricular time-activity curve by use of a previously validated method28 in which corrections were made for the limited recovery of the left ventricular ROI and the spillover from the myocardial signals.

Coronary flow reserve was defined as the ratio of myocardial blood flow after dipyridamole infusion to that at baseline. In addition, coronary resistance values were calculated both at baseline and after dipyridamole infusion by dividing mean arterial blood pressure by the respective myocardial flow value.

**Analytical Procedures**

All venous blood samples were taken from subjects after 12 hours of fasting. Serum samples were stored frozen at $-70^\circ\text{C}$ until analyzed. Serum total cholesterol and TG were determined with automated enzymatic methods.29 Serum apoB was measured by an immunochemical assay (Orion Diagnostica). Lipoprotein fractions, LDL, HDL, and serum apo concentrations were determined by an immunoturbidimetric method with a commercially available kit (Boehringer Mannheim) based on sequential flotation as described.30 Familial hypercholesterolemia was excluded from each pedigree by determining the LDL receptor status of the proband by use of the lymphocyte culture method.31

**Statistical Analysis**

Results are presented as mean±SD. Comparisons between the 2 groups were performed by the t test or the Wilcoxon rank-sum test as appropriate. Spearman’s correlation coefficients were calculated to study the associations between flow reserve and lipid variables. All statistical analyses were performed with the SAS statistical program package (SAS Institute Inc).

**Results**

**Subject Characteristics**

The characteristics of the subjects are shown in Tables 1 and 2. There were no differences in age ($34.8\pm5.4$ versus $35.5\pm4.0$ years, $P=\text{NS}$) between the FCHL patients and control subjects; body mass index (BMI) was slightly higher in the FCHL group ($26.3\pm2.9$ versus $24.7\pm2.0$ kg/m$^2$, $P<0.05$). Serum total cholesterol ($7.0\pm0.6$ versus $4.8\pm0.8$ mmol/L, $P<0.05$), LDL

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**TABLE 2. Characteristics and Flow Results of Control Subjects**

<table>
<thead>
<tr>
<th>Subject</th>
<th>Age, y</th>
<th>BMI, kg/m$^2$</th>
<th>TC, mmol/L</th>
<th>LDL-C, mmol/L</th>
<th>HDL-C, mmol/L</th>
<th>HDL-C/TC</th>
<th>TG, mmol/L</th>
<th>Basal Flow, mL · min$^{-1} · g^{-1}$</th>
<th>Dipyridamole Flow, mL · min$^{-1} · g^{-1}$</th>
<th>Flow Reserve</th>
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<td>0.7</td>
<td>0.98</td>
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<td>5.71</td>
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<td>0.77</td>
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<td>0.73</td>
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<td>0.81</td>
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<td>0.7</td>
<td>0.68</td>
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Abbreviations as in Table 1.
TABLE 3. Hemodynamic Data During PET Scan

<table>
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<tr>
<th></th>
<th>Heart Rate, bpm</th>
<th>Systolic Blood Pressure, mm Hg</th>
<th>Diastolic Blood Pressure, mm Hg</th>
<th>Rate-Pressure Product (Systolic), mm Hg·min⁻¹</th>
<th>Change, mm Hg·min⁻¹</th>
<th>bpm</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Rest</td>
<td>Dipy</td>
<td>Rest</td>
<td>Dipy</td>
<td>Rest</td>
<td>Dipy</td>
</tr>
<tr>
<td>FCHL (n=21)</td>
<td>60±9</td>
<td>67±11*</td>
<td>124±13</td>
<td>128±15</td>
<td>70±8</td>
<td>71±9</td>
</tr>
<tr>
<td>Control subjects</td>
<td>61±7</td>
<td>84±10*</td>
<td>124±14</td>
<td>126±13</td>
<td>71±11</td>
<td>73±10</td>
</tr>
</tbody>
</table>

Dipy indicates after dipyridamole administration. Values are mean±SD.

*P<0.05 between baseline and after dipyridamole administration.

Serum total cholesterol (7.0±0.14 versus 3.0±0.7 mmol/L, P<0.05), apoB (1.38±0.14 versus 0.89±0.21 g/L, P<0.001), and TG (2.3±0.9 versus 1.4±0.9 mmol/L, P<0.05) concentrations were significantly higher in the FCHL patients compared with control subjects. There were no differences in serum HDL cholesterol concentrations (1.29±0.35 versus 1.38±0.21 mmol/L, P=NS).

The FCHL phenotype IIA and IIB patients had similar serum total cholesterol (7.0±0.6 versus 7.1±0.5 mmol/L, P=NS), apoB (1.33±0.14 versus 1.45±0.13 g/L, P=NS), and LDL cholesterol (4.8±0.7 versus 4.5±0.5 mmol/L, P=NS) concentrations. Phenotype IIB patients had significantly higher TG (3.26±0.82 versus 1.76±0.40 mmol/L, P<0.05) and lower HDL cholesterol concentrations (1.0±0.22 versus 1.41±0.35 mmol/L, P<0.05) and lower ratios of HDL cholesterol to total cholesterol (0.16±0.03 versus 0.20±0.05, P<0.05) compared with the phenotype IIA group.

Additionally, BMI was higher in FCHL patients with phenotype IIB compared with phenotype IIA (28±3.7 versus 25.3±2.0 kg/m², P<0.05).

### Hemodynamic Parameters During PET

Dipyridamole administration induced significant increases in heart rate and rate-pressure product in both subject groups (Table 3). There were no differences between the FCHL patients and control subjects in heart rate and systolic or diastolic blood pressures either at baseline or during maximal vasodilation. Accordingly, the rate-pressure products were similar between the groups.

### Myocardial Blood Flow

Basal myocardial blood flow was similar in the FCHL patients and control subjects (0.79±0.19 versus 0.88±0.20 mL·g⁻¹·min⁻¹, P=NS). A significant increase in flow was obtained in both subject groups by dipyridamole infusion, but the flow at maximal vasodilation was significantly lower in FCHL patients compared with control subjects (3.54±1.59 versus 4.54±1.17 mL·g⁻¹·min⁻¹, P=0.025) (Figure 1). The difference in coronary flow reserve values between patients and control subjects did not reach statistical significance (4.7±2.2 versus 5.3±1.6, P=NS), but the frequency of low coronary flow reserve values appeared to be greater in the FCHL patients compared with control subjects (Figure 2).

The estimated total coronary resistance was slightly higher at baseline in the FCHL group compared with control subjects, but the difference was not statistically significant (118±31 and 104±22 mm Hg·min⁻¹·g⁻¹·mL⁻¹ in patients and control subjects, P=0.097). During hyperemia, coronary resistance was clearly reduced in both groups but remained significantly higher in the FCHL patients than in control subjects (31±15 versus 23±12 mm Hg·min⁻¹·g⁻¹·mL⁻¹, P=0.017).

When the FCHL group was divided according to phenotype (IIA, n=13; IIB, n=8), the basal myocardial blood flow was similar (0.79±0.14 versus 0.79±0.27 mL·g⁻¹·min⁻¹) between the subgroups, but significant differences were found in hyperemic flow (4.2±1.5 versus 2.5±1.2 mL·g⁻¹·min⁻¹, P<0.05), coronary flow reserve (5.4±2.0 versus 3.4±2.1, P<0.05), and coronary resistance during hyperemia (25±11 versus 41±16 mm Hg·min⁻¹·g⁻¹·mL⁻¹, P<0.05) between phenotypes IIA and IIB, respectively (Figure 3).

Figure 4 shows the relationship between cholesterol concentration and coronary resistance during hyperemia. Higher cholesterol was generally associated with higher resistance (r=0.38, P=0.014), although a considerable number of FCHL patients with high actual cholesterol concentration exhibited normal coronary resistance values. Most patients having high coronary resistance were classified as FCHL phenotype IIB. In contrast, most patients with low resistance were phenotype IIA patients.

In the pooled population, total cholesterol (r=-0.33, P<0.05) and TG (r=-0.35, P<0.05) concentrations correlated significantly with myocardial flow during hyperemia. However, in study groups separately or in FCHL subgroups, no significant associations between lipid values and coronary reactivity parameters were detected.

### Discussion

The present study shows that abnormalities in coronary flow and its regulation exist in young male patients with FCHL.
Furthermore, we found considerable heterogeneity in coronary reactivity within the FCHL group that was related to lipid phenotype. In patients with phenotype IIB in whom both cholesterol and TG levels were elevated, the hyperemic flow was 45% lower than in control subjects. In patients with phenotype IIA, however, hyperemic flow was similar to that seen in healthy subjects, despite their high total cholesterol concentration.

Dyslipidemia is a major risk factor for coronary artery disease (CAD). There is much evidence linking elevated LDL cholesterol and low HDL cholesterol to CAD.32 The relation between plasma TG and CAD is not as well established.33 Recently, however, Jeppesen et al34 found that fasting hypertriglyceridemia was a strong predictor of coronary heart disease independent of other risk factors, including HDL cholesterol. In addition, currently unknown factors are important determinants for CAD because the currently recognized risk factors explain only up to 60% of coronary mortality.35 In our previous studies with FH patients20 and healthy subjects,22,23 coronary reactivity was associated with LDL but not with TG concentration. The high incidence of premature CAD in FCHL patients has been related to their lipoprotein abnormalities; increased concentration of small VLDL and small, dense LDL; and decreased concentration of HDL.1,36,37 The present study did not include FCHL patients with phenotype IV (isolated hypertriglyceridemia). Therefore, the higher TG concentrations and lower HDL cholesterol values were associated with phenotype IIB. The preserved coronary reactivity in phenotype IIA patients with relatively high cholesterol concentrations is in contrast with findings in FH patients in whom a linear relationship between coronary reactivity and cholesterol values was detected.20,37 However, the genetic background and thus the metabolic pathophysiology are entirely different between FCHL and FH. While the major genes causing FCHL remain undiscovered, the detailed mechanisms causing premature atherosclerosis in affected FCHL family members are not known. The narrowing of the candidate gene area to chromosome 1q21–23 and the exclusion of lipid genes from that region indicate that a novel gene is probably a major cause of FCHL.12 Our genetic linkage analyses were performed with emphasis on lipid phenotype IIB. It was of particular interest that the most marked abnormalities in coronary reactivity were also observed in subjects with phenotype IIB. Thus, the genetic factors behind FCHL may cause endothelial and/or smooth muscle dysfunction by mechanisms unrelated to lipid metabolism, which may in part explain the lack of correlation between coronary reactivity and TG or HDL cholesterol in FCHL patients. Because the critical linkage area in chromosome 1 was only 7 to 8 centimorgans, the FCHL gene will be cloned in the near future.

Previously, abnormal coronary flow response to acetylcholine or dipyridamole has been demonstrated in a variety of pathologic settings such as CAD,38 syndrome X,39 and hypertrophic cardiomyopathy.40 In CAD patients, abnormalities in flow response to vasodilating agents have been found in mildly stenosed41 and even nonstenosed coronary arteries.42 Dayanikli et al19 have used PET to study asymptomatic middle-aged subjects with a family history of CAD and high-risk lipid profiles. Reduced coronary flow reserve was detected in the high-risk patients compared with the control.

Figure 2. Coronary flow reserve in control subjects and FCHL patients. Displayed are distributions of flow reserve values in both subject groups. Bottom of vertical line marks 5th percentile. Bottom of box marks 25th percentile; median line marks 50th percentile; top of box marks 75th percentile; and top of vertical line marks 95th percentile. Symbol ■ in box marks mean; •, minimum value and 0 percentile; and ● above vertical line, maximum value.

Figure 3. Coronary flow at baseline and during hyperemia in FCHL phenotype groups. Significantly lower hyperemic flow values were detected in FCHL phenotype IIB patients (*P<0.05).

Figure 4. Relationship between hyperemic coronary resistance and serum total cholesterol concentration. Resistance values were associated with actual serum cholesterol concentrations, but there was considerable variability of resistance values at given cholesterol concentrations, and phenotype IIB appears to show higher values.
group. In addition to our findings about abnormalities in flow reserve in young men with FH and in patients with minimally complicated IDDM, we have observed an association between flow reserve and risk factors for CAD in healthy young men.

In this study, we used dipyridamole as a vasodilating agent to test coronary function. Dipyridamole increases interstitial adenosine concentration in vascular smooth muscle, leading to relaxation of coronary resistance vessels. It has been postulated that increased shear stress associated with increased flow will induce the release of vasodilating substances from endothelial cells and thus elicit more prominent vasodilatation in vessels with preserved endothelial function. Indeed, coronary flow response to dipyridamole or adenosine has been found to relate to endothelium-dependent vasodilatation. Thus, coronary flow response to dipyridamole or adenosine can be regarded as an integrating measure of endothelial function and vascular smooth muscle relaxation.

Only male patients with FCHL were enrolled in this study. We do not know whether the same results can be extrapolated to female subjects of a similar age. In the present study, only FCHL phenotypes IIA and IIB were studied. Thus, patients with other phenotypes were not represented in this study, and it is unclear whether the results are applicable to the other FCHL phenotypes.

Conclusions

The present study shows that abnormalities in coronary flow and its regulation exist in young male adults with FCHL. The abnormalities appear to be less extreme in FCHL patients than obtained in the other hereditary lipid disorder, familial hypercholesterolemia. However, our results show considerable heterogeneity in vascular reactivity within FCHL. The FCHL phenotype IIB with elevations in both serum cholesterol and its regulation exist in young male adults with FCHL. The present study shows that abnormalities in coronary flow reserve in healthy young men.

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