CARDIOVASCULAR MEDICINE

Regional myocardial perfusion defects during exercise, as assessed by three dimensional integration of morphology and function, in relation to abnormal endothelium dependent vasoreactivity of the coronary microcirculation

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Objective: To test the hypothesis that scintigraphic regional myocardial perfusion defects during exercise in patients with normal coronary angiography may be related to abnormal endothelium dependent vasoreactivity of the corresponding myocardial territory in response to cold pressor testing.
Methods: 38 patients were classified into two groups according to the presence or absence of exercise induced scintigraphic myocardial perfusion defects. A cold pressor test was done in all patients during routine coronary angiography, followed by dynamic positron emission tomography to establish coronary blood flow mediated vasoreactivity of the epicardial coronary artery and the myocardial territories supplied by the left anterior descending, left circumflex, and right coronary arteries.
Results: 28 patients had regional myocardial perfusion defects while 10 had normal scintigraphic imaging. The three dimensional scintigraphic fusion image revealed 49 regional myocardial perfusion defects with a mean (SD) reversibility of the original stress defect of 20 (3)%. In patients with exercise induced regional myocardial perfusion defects, the responses of epicardial luminal area and regional myocardial blood flow (RMBF) to cold pressor testing were reduced compared with patients with normal perfusion imaging (epicardial luminal area: 5.2 (1.2) to 4.2 (0.86) mm^2 *v* 4.7 (0.5) to 5.8 (0.5) mm^2; RMBF: 0.75 (0.16) to 0.78 (0.20) ml/g/min *v* 0.75 (0.15) to 1.38 (0.26) ml/g/min; $p \leqslant 0.03$, respectively). In patients with regional abnormal scintigraphic perfusion, the corresponding RMBF response to cold pressor testing was more severely impaired than the mean myocardial blood flow in the remaining two vascular territories, but the difference was not significant (0.75 (0.16) to 0.78 (0.20) ml/g/min *v* 0.75 (0.10) to 0.87 (0.12) ml/g/min; NS). The endothelium independent increase in RMBF induced by glyceryl trinitrate did not differ between patients with exercise induced myocardial perfusion defects and those with normal perfusion images (0.75 (0.16) to 0.94 (0.09) ml/g/min *v* 0.75 (0.15) to 0.94 (0.09) ml/g/min; NS). There was a highly significant correlation between the endothelium dependent responses of RMBF to cold pressor testing and the severity of exercise induced scintigraphic regional myocardial perfusion defects ($r = 0.95$, $p = 0.001$).
Conclusions: Exercise induced scintigraphic regional myocardial perfusion defects in patients with angina but normal coronary angiography may be related to abnormal endothelium dependent vasoreactivity of the corresponding myocardial territory.

Chest pain in patients without angiographically significant obstructive coronary artery lesions has been recognised as a common problem encountered in clinical practice.[1 2] According to the coronary artery surgery study (CASS) more than 50% of women and 17% of men with chest pain referred for coronary angiography do not have angiographically significant obstructive coronary artery disease.[3] Although non-cardiac causes may account for the chest pain syndrome, myocardial ischaemia in the absence of angiographically significant coronary artery stenoses has been suspected as a possible cause.[4] This has led to the new concept that endothelial dysfunction of the coronary circulation may be responsible for myocardial ischaemia during stress testing.[5]

The vascular endothelium plays a central role in maintaining vasomotor tone by releasing endothelium dependent vasoconstricting and vasodilating factors. Endothelium derived relaxing factors (EDRF), such as prostacyclin, hyperpolarising relaxing factor, and nitric oxide, are mainly responsible for the maintenance of myocardial blood flow during increased metabolic demand.[6–9] However, risk factors for atherosclerosis have been shown to impair the activity of EDRF, thereby affecting the neurohumoral dynamic balance of the vascular wall in favour of flow mediated, endothelium dependent coronary vasoconstriction.[6 10 11] Indeed, clinical studies[5 12] have shown that endothelial dysfunction of the coronary arteriolar vessels in response to acetylcholine may be associated with scintigraphic myocardial perfusion defects of the anterior wall. However, there has not been a comprehensive three dimensional evaluation of exercise induced regional myocardial perfusion defects in patients with normal coronary

Abbreviations: ANOVA, analysis of variance; CASS, coronary artery surgery study; EDRF, endothelium derived relaxing factors; LDL, low density lipoprotein; PET, positron emission tomography; RMBF, regional myocardial blood flow; SPECT, single photon emission computed tomography; VLDL, very low density lipoprotein

Table 1 Characteristics of the study population

Characteristic	Smokers	Hypercholesterolaemic	Hypertensive	Controls
Number	10	10	8	10
Age (years)	53 (2)	56 (4)	54 (4)	57 (3)
Sex (male/female)	6/4	5/5	5/4	4/5
Lipid profile				
Total cholesterol (mmol/l)	5.34 (0.26)*	6.81 (0.26)*	4.97 (0.18)	4.14 (0.05)
LDL (mmol/l)	3.63 (0.16)*	4.35 (0.21)*	2.90 (0.21)	2.41 (0.21)
VLDL (mmol/l)	0.62 (0.08)	1.04 (0.31)*	0.67 (0.08)	0.49 (0.10)
HDL (mmol/l)	1.27 (0.08)	1.19 (0.13)	1.30 (0.08)	1.55 (0.08)
Triglyceride (mmol/l)	1.85 (0.23)	1.76 (0.44)	1.13 (0.14)	0.99 (0.12)
Fibrinogen (g/l)	2.97 (0.17)	2.90 (0.11)	3.10 (0.23)	2.58 (0.22)
Haemodynamics				
Heart rate (beats/min)	60 (2)	63 (2)	63 (2)	58 (4)
Blood pressure (mm Hg)				
Systolic	116 (3)	120 (5)	152 (2)*	126 (6)
Diastolic	72 (3)	72 (3)	82 (2)*	70 (3)
BMI (kg/m^2)	22.8 (1.8)	23.3 (1.7)	24.8 (2.6)	22.8 (1.0)
LVMI (g/m^2)	84 (9)	85 (5)	104 (4)*	76 (6)

Values are n or mean (SD).
*$p \leq 0.05$ *v* control.
BMI, body mass index; HDL, high density lipoprotein; LDL, low density lipoprotein; LVMI, left ventricular mass index; VLDL, very low density lipoprotein.

angiography but abnormal endothelium dependent vasomotion of the corresponding myocardial territory in response to a physiological stimulus such as cold pressor testing. To do this, an image fusion technique providing a three dimensional reconstructed coronary artery tree and a three dimensional myocardial scintigraphic perfusion image can be used to obtain accurate information about regional myocardial territories and the corresponding vessel segments.[13–15]

Our aim in this study was to test the hypothesis that exercise induced scintigraphic regional myocardial perfusion defects in patients with normal coronary angiography may be related to abnormal blood flow mediated vasoreactivity of the corresponding myocardial territory in response to cold pressor testing.

METHODS

Patient population

The study population included 38 patients studied prospectively who underwent both thallium-201 (^{201}Tl) scintigraphic perfusion imaging during physical exercise and subsequent routine diagnostic catheterisation for the evaluation of chest pain. The patients were classified into two groups according to the presence or absence of exercise induced ^{201}Tl myocardial perfusion defects suggestive of myocardial ischaemia.[16] Those with exercise induced ^{201}Tl myocardial perfusion defects were classified according to their risk factors for atherosclerosis (10 chronic smokers aged 53 (2) years (mean (SD)), 10 hypercholesterolaemic patients aged 56 (4) years, and eight hypertensive patients aged 54 (4) years), while 10 patients with normal ^{201}Tl myocardial perfusion during exercise and without known risk factors for atherosclerosis (mean age of 57 (3) years) served as controls (table 1).

A prerequisite for inclusion in the study was the absence of significant coronary artery stenoses. Patients with evidence of coronary myocardial bridging on coronary angiography and those with previous Q wave or non-Q wave myocardial infarction but angiographically normal coronary arteries were not included in the study. Other cardiac and non-cardiac diseases were excluded after full clinical, laboratory, and routine echocardiographic evaluation. In order to avoid possible confounding effects of medical treatment on endothelium dependent vasoreactivity, patients were not included if they were on vasoactive drugs such as angiotensin converting enzyme inhibitors, calcium channel blockers, or β-hydroxymethylglutaryl-coenzyme A reductase inhibitors at the time of the study. β Blockers and diuretics were allowed for blood pressure control.

In each patient, quantitative angiographic evaluation was undertaken at baseline and during cold pressor testing to establish blood flow mediated vasoreactivity of the epicardial coronary artery corresponding to the myocardial region involved in exercise induced ^{201}Tl perfusion defects.[17] At the time of selective coronary angiography, normal endothelium dependent vasoreactivity was defined as blood flow mediated vasodilatation of the vessel, whereas the absence of flow mediated vasodilatation or even vasoconstriction during cold pressor testing was regarded as abnormal endothelium dependent coronary vasomotion.[10 18] The maximum endothelium independent vasodilator response of the epicardial coronary artery was tested by the intracoronary administration of 0.2 mg of glyceryl trinitrate. All 38 patients had angiographically normal, smooth appearing coronary arteries. Thirteen patients had minor luminal irregularities (involving the left circumflex coronary artery in six, the right coronary artery in four, and the left anterior descending coronary artery in three), but not in the vessel under study. Subsequently, in each patient endothelium dependent responses of regional myocardial blood flow (RMBF) to cold pressor testing were determined by dynamic positron emission tomography (PET).[19] The endothelium independent glyceryl trinitrate induced increase in RMBF was assessed by dynamic PET after sublingual application of 0.8 mg glyceryl trinitrate.

Patients with unstable angina, recent myocardial infarction, a clinical history suggestive of variant angina, valvar heart disease, clinical evidence of heart failure, or diabetes mellitus were excluded. No patient had angiographic or echocardiographic evidence of left ventricular hypertrophy. Left ventricular end diastolic pressure was within the normal range ($\leq$ 12 mm Hg). All patients were in sinus rhythm and none had ECG evidence of left bundle branch block, significant Q waves, or alterations of the ECG that could interfere with reliable analysis of ST segment changes. In addition, no patient had perfusion abnormalities on ^{201}Tl perfusion imaging at rest indicative of previous myocardial infarction. β Blockers and diuretics were discontinued at least 24 hours before ^{201}Tl perfusion imaging and during the subsequent period of investigation.

All patients gave written informed consent before the study. The study protocol and amount of radiation exposure involved in the scintigraphic techniques (about 6.7 mS) were approved by the local ethics committee of the University of Freiburg.

Treadmill exercise testing

All patients underwent a symptom limited treadmill exercise test in the morning, according to a modified Bruce protocol, under continuous ECG monitoring. A 12 lead standard ECG was recorded and blood pressure measured at the onset of the test, at the end of each stage, at peak exercise, at 1 mm ST segment depression and at angina (when they occurred), and in any cases when clinically indicated. The exercise test was terminated when one of the following end points was reached: physical exhaustion; progressive angina; ST segment depression $\geqslant 3$ mm; or the occurrence of clinically harmful conditions. A significant ST segment depression was considered as a horizontal or downsloping ST segment deviation of $\geqslant 1$ mm at 0.08 seconds after the J point. The occurrence of ST segment depression and typical or atypical angina was documented.

Exercise ^{201}Tl single photon emission computed tomographic imaging

All patients underwent thallium scintigraphy while exercising on an electronically braked bicycle in the supine position with the workload starting at 50 W and increasing by 25 W every three minutes. Criteria for exercise end points and significant ST segment depression were the same as in the bicycle exercise test. Any cardiac and vasoactive drugs were withdrawn at least 12 hours before exercise testing. At peak exercise, 2–3 mCi (70–105 MBq) ^{201}Tl was given intravenously and the patients continued to exercise for an additional 60 seconds. Redistribution images were acquired four hours after exercise testing while the patients were resting. The myocardial perfusion data were acquired with single photon emission computed tomography (SPECT) using standard acquisition protocols. SPECT images were obtained with a large field of view rotating camera (Orbiter and Diacam, Siemens, Erlangen, Germany; or Prism XP 3000, Picker, Cleveland, Ohio, USA).

Quantitative coronary angiography

Selective coronary angiography was undertaken using a biplane, isocentric multidirectional digital angiographic system (BICOR-HICOR, Siemens). End diastolic images of coronary arteries were evaluated quantitatively by automatic contour detection.[17] In all 38 patients, quantitative measurements were undertaken in a selected 4–8 mm long relatively straight left anterior descending coronary artery segment (in 20 patients) or left circumflex coronary artery segment (in 18 patients), and special care was taken to avoid overlap of the coronary segments. Tapered or tortuous segments were not used. In all patients, measurements of the corresponding segments of interest were performed in both views of the biplane images, using the take-off points of side branches as anatomical landmarks for identification of corresponding vessel segments. The luminal area of the vessel segments was analysed from biplane views, assuming an elliptical shape; these measurements were done at baseline, after cold pressor testing, and after glyceryl trinitrate application.[17] Owing to the selection criteria for the vessel segments, the segments finally analysed were not necessarily the most constricting segments.

Positron emission tomography

Regional myocardial blood flow was measured with ^{13}N labelled ammonia, using PET and a previously validated two compartment model.[19] Patients were positioned in a 951 Siemens ECAT positron camera. Myocardial blood flow was measured at baseline and during cold pressor testing. Starting with the administration of intravenous ^{13}N labelled ammonia (15–20 mCi), serial transaxial emission images were acquired (12 image frames of 10 seconds each, two frames of 30 seconds each, and one frame of 900 seconds). Cold pressor testing involved the subject immersing the left hand into ice water for 30 seconds before a second dose of ^{13}N labelled ammonia (15–20 mCi) was injected. The image acquisition sequence used for the baseline study was repeated during a two minute period of cold pressor testing to permit trapping of ^{13}N labelled ammonia in the myocardium. Heart rate, arterial blood pressure, and a 12 lead ECG were recorded continuously.

RMBF was quantified from the territories of the left anterior descending, left circumflex, and right coronary arteries on three mid-ventricular slices. In addition, the RMBF values of the remaining two vascular territories were averaged to calculate the mean myocardial blood flow. A small region of interest (25 mm^2) was centred in the left ventricular blood pool for determining the arterial input function.

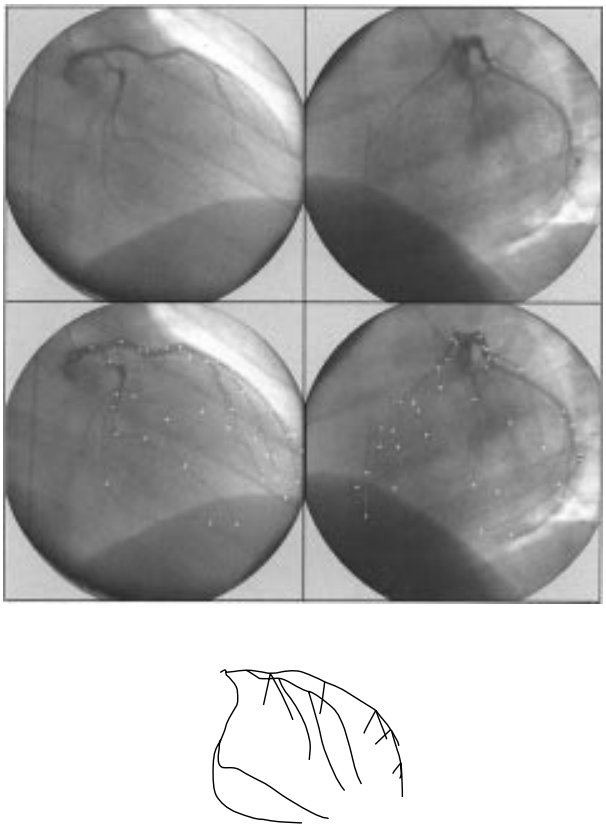

Figure 1 Left coronary angiogram: right and left anterior oblique views (RAO, LAO) from a patient without significant obstructive coronary artery disease in the left coronary artery, from which the three dimensional coronary tree structure was calculated (upper panels). The middle panels show RAO and LAO superimposition of the reprojected three dimensional models onto the angiograms from the same views. The lower panels show views of the computed three dimensional structure of the left and right coronary trees.

Three dimensional scintigraphic myocardial fusion image

Three dimensional scintigraphic myocardial fusion imaging has been described in detail previously.[13–15] The three dimensional coronary artery tree structure was reconstructed from two simultaneously acquired angiographic views.[20] The anatomical course of the vessel was segmented by three dimensional Bézier curves, and the three dimensional

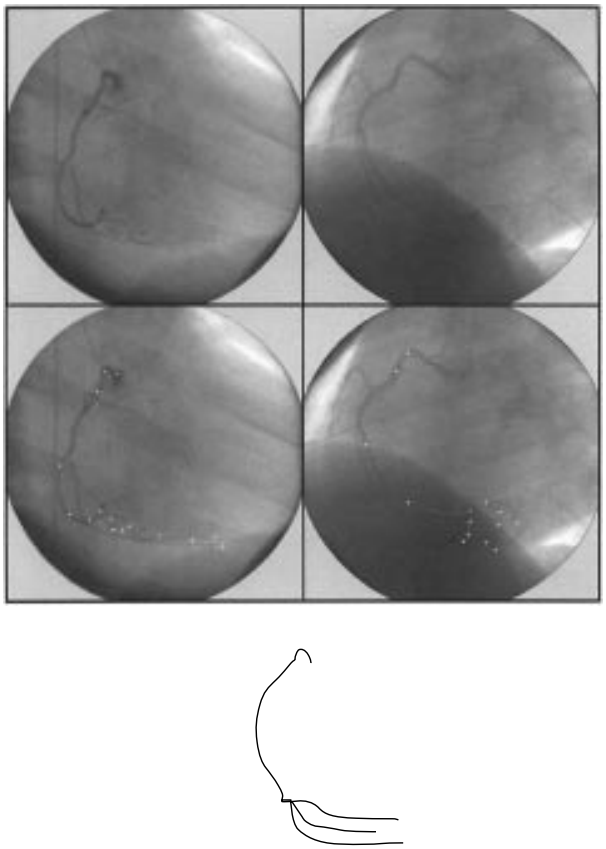

Figure 2 Right coronary angiogram: right and left anterior oblique views (RAO, LAO) from a patient without significant obstructive coronary artery disease in the right coronary artery, from which the three dimensional coronary tree structure was calculated (upper panels). The middle panels show RAO and LAO superimposition of the reprojected three dimensional models onto the angiograms from the same views. The lower panels show views of the computed three dimensional structure of the left and right coronary trees.

structure of the coronary tree was computed from a set of 20–30 Bézier curves (figs 1 and 2). The relative location of the right coronary ostium in relation to the left coronary ostium, which could be determined by routine ventriculography, allowed a combined three dimensional display of both right and left coronary trees. Three dimensional reconstruction of the scintigraphic myocardial surface was done by assessing the position of the left ventricle in cubic voxels obtained from maximum activity points on spherically distributed rays originating from four central points of the ventricle. For image fusion, the meridian coordinates of the scintigraphic myocardial surface were shifted to corresponding values of the coronary tree.

Image interpretation

A reversal of 15% or more of the original stress defect was defined as representing a significant myocardial perfusion defect.[16] Interpretation of the three dimensional scintigraphic fusion image was achieved by consensus between two experienced investigators unaware of the clinical history, the subsequent coronary angiography, or the results of vasomotor testing of the coronary circulation. Myocardial scintigraphic perfusion on the three dimensional fusion image was evaluated semiquantitatively by analysing per cent maximum activity of the corresponding myocardial regions. Three dimensional scintigraphic image fusion with the reconstructed coronary tree structure allowed an accurate assignment of coronary vessel segments to the corresponding myocardial perfusion regions.[13–15]

Statistical analysis

For descriptive purpose all data are presented as mean (SD), with relative frequencies where calculated. Differences of baseline values between groups were tested using the Wilcoxon rank test for quantitative variables or the χ^2 test for qualitative variables. Relative changes in cold pressor test induced vascular responses within each group were analysed using the Wilcoxon sign rank test. Comparisons of these changes between the different study groups were investigated by the two sample Wilcoxon rank test. Correlations between selected variables were estimated by Spearman correlation coefficients. All test procedures were two sided, and a probability value of $p < 0.05$ indicates significance.

RESULTS

Clinical characteristics

The clinical characteristics of the study population are given in table 1. Ten patients had normal exercise thallium tests, whereas in 28 patients (10 chronic smokers, 10 patients with hypercholesterolaemia, and eight with hypertension) exercise thallium testing revealed regional myocardial perfusion defects suggestive of myocardial ischaemia.

As a group the chronic smokers reported a history of 32 (9) pack-years. Total serum cholesterol and low density lipoprotein (LDL) cholesterol values in the smokers were within the normal range, but significantly higher than in control group (5.34 (0.26) *v* 4.14 (0.31) mmol/l and 3.63 (0.16) *v* 2.41 (0.21) mmol/l, respectively; $p \leq 0.05$).

The baseline characteristics of hypercholesterolaemic patients and controls were similar apart from the lipid profile. In the hypercholesterolaemic patients, the total cholesterol, LDL cholesterol, and very low density lipoprotein (VLDL) cholesterol concentrations were significantly higher than in the control group, at 6.81 (0.26) *v* 4.14 (0.31) mmol/l for total, 4.35 (0.21) *v* 2.41 (0.21) mmol/l for LDL, and 1.04 (0.31) *v* 0.49 (0.10) mmol/l for VLDL ($p \leq 0.05$ for each comparison).

In the hypertensive group, one patient was receiving combined medical treatment with β blockers and diuretics, one patient with β blockers alone, and two patients with diuretics alone, while four patients were not receiving drug treatment. However, each hypertensive patient had a well established history of chronically raised blood pressure ($\geq$ 150/95 mm Hg) without any apparent underlying cause, and their total cholesterol concentrations were within the normal range (4.97 (0.18) mmol/l). The left ventricular mass index in the hypertensive patients was also within the normal range, but was significantly higher than in either the smokers, the hypercholesterolaemic patients, or the controls (104 (4) *v* 84 (9) *v* 85 (5) *v* 76 (6) g/m^2, respectively; $p \leq 0.05$).

Bicycle exercise testing

Significant ST segment depression was induced during exercise in 20 of the 28 patients with regional myocardial perfusion defects (71%) (group A), whereas two of the eight patients with normal perfusion (25%) (group B) had significant ST segment changes. The location and radiation of pain were judged typical of a cardiac ischaemic origin in 25 patients from group A (89%) and in four patients from group B (50%). The other four patients from group B reported more atypical findings, such as prolonged duration of pain, occurrence of pain at rest, and poor benefit from sublingual nitrates. Although some patients in group B had significant ST segment depression (two patients) and anginal chest pain (four patients), no regional ischaemic perfusion defects were found during exercise.

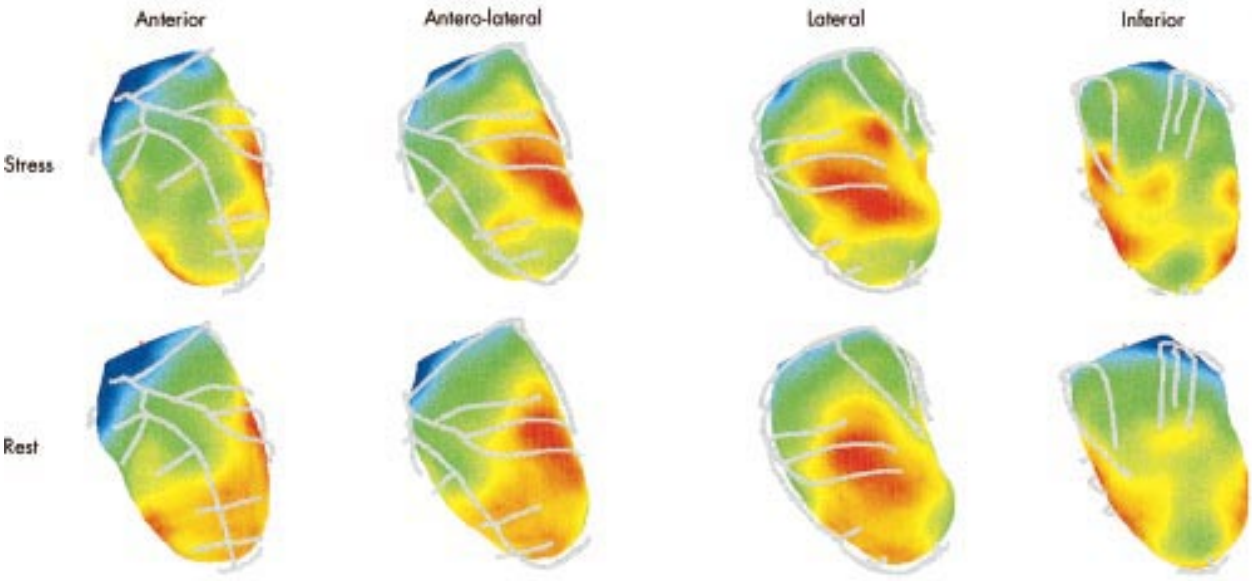

Figure 3 Visualisation of the combined three dimensional display of morphological data and regional myocardial perfusion data from four different viewing angles during stress and rest.

Myocardial perfusion defects on three dimensional scintigraphic fusion imaging

In 38 patients we identified a total of 49 regional myocardial perfusion defects during exercise on the three dimensional scintigraphic fusion image. Twelve myocardial perfusion defects (25%) during exercise were ascribed to the left anterior descending coronary artery, 10 (21%) to the right coronary artery, nine (18%) to the left circumflex coronary artery, seven (14%) to the diagonal branch, four (8%) to the posterolateral branch, four (8%) to the obtuse marginal branch, and three (6%) to the septal branch. The mean reversibility of the original stress defect was 20 (3)%. In 10 patients no regional myocardial perfusion defects were identified.

A representative example is shown in figs 1, 2, and 3. Coronary angiography was undertaken in right anterior oblique (RAO) and left anterior oblique (LAO) views (figs 1 and 2, upper panels). In this patient, no significant obstructive coronary artery lesion is seen. The angiographic course of the vessels is segmented by Bézier curves superimposed in each projection plane (figs 1 and 2, middle panels). From these Bézier curves, the three dimensional structure of the coronary tree is computed (figs 1 and 2, lower panels). The three dimensional myocardial perfusion image is reconstructed from the original data. In this patient the scintigraphic fusion image (fig 3) showed a reversible perfusion defect in the anterior wall and apex during stress, with an accurate assignment to the left anterior descending coronary artery, while there was normal perfusion in the lateral and inferolateral walls supplied by an obtuse marginal branch and by the left circumflex coronary artery. In addition, normal myocardial perfusion during stress testing was found in the inferior wall, corresponding to the right coronary artery. The regional myocardial territory of each coronary vessel is clearly displayed on the three dimensional fusion image.

Responses of the target related epicardial artery to cold pressor testing and glyceryl trinitrate (all patients)

Table 2 shows the mean luminal area of all coronary segments analysed at baseline, during cold pressor testing, and after intracoronary glyceryl trinitrate. In 28 patients with exercise induced regional myocardial perfusion defects, classified according to their risk factors for atherosclerosis, cold pressor testing led to a significant decrease ($p \leq 0.002$) in the luminal area of the epicardial coronary artery (5.2 (1.2) to 4.2 (0.86) mm^2) compared with 10 patients with normal perfusion images (4.7 (0.5) to 5.8 (0.5) mm^2).

Figure 4 shows the individual responses of the coronary segments to cold pressor testing for each group. In chronic smokers with exercise induced regional myocardial perfusion defects, cold pressor testing caused a decrease in luminal area from 5.9 (1.6) to 4.2 (1.1) mm^2 ($p \leq 0.002$). In the hypercholesterolaemic and hypertensive patients with exercise induced regional myocardial perfusion defects, a significant decrease in luminal area from 5.0 (0.9) to 4.3 (0.7) mm^2 and from 4.6 (0.9) to 4.2 (0.9) mm^2, respectively, was observed in response to cold pressor testing (both $p \leq 0.02$). In contrast, 10 patients referred for coronary angiography with normal perfusion images during exercise showed normal endothelium dependent vasomotion, with a cold pressor test induced increase in mean luminal area from 4.7 (0.5) to 5.8 (0.5) mm^2 ($p \leq 0.002$). Group comparison of the cold pressor test induced decrease in mean luminal area between patients with and without exercise induced perfusion defects showed a significant difference ($p \leq 0.03$). Endothelium independent vasodilatation of epicardial coronary arteries induced by glyceryl trinitrate did not differ between patients with exercise induced perfusion defects and those with normal perfusion images (table 2).

Vascular responses of regional myocardial blood flow to cold pressor testing and glyceryl trinitrate

In patients with exercise induced regional myocardial perfusion defects, the responses of corresponding RMBF to

Table 2 Responses of luminal area to cold pressor testing and glyceryl trinitrate in patients with exercise induced regional myocardial perfusion defects and in patients with normal myocardial perfusion (controls)

Group	Baseline (mm^2)	CPT (mm^2)	GTN (mm^2)
Smokers	5.9 (1.6)	4.2 (1.1)*	7.6 (1.5)
Hypercholesterolaemic	5.0 (0.9)	4.3 (0.7)*	6.9 (2.1)
Hypertensive	4.6 (0.9)	4.2 (0.9)*	6.1 (1.3)
Controls	4.7 (0.5)	5.8 (0.5)*	6.3 (1.5)

Values are mean (SD).
*$p \leq 0.02$ *v* corresponding baseline.
CPT, cold pressor test; GTN, glyceryl trinitrate.

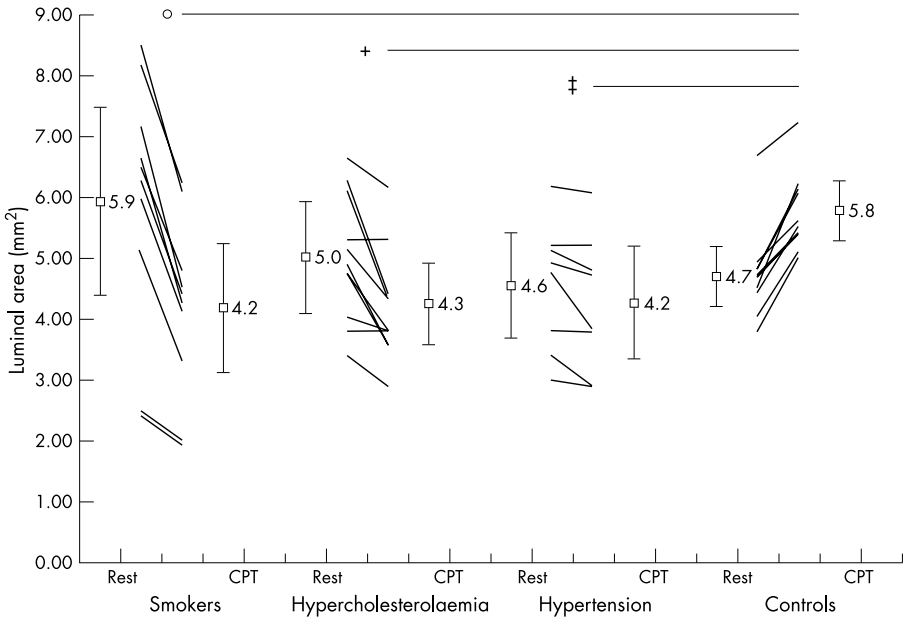

Figure 4 Individual responses of the analysed coronary segments to cold pressor testing (CPT) from baseline in four groups of patients. Endothelium dependent vasomotion of the epicardial coronary artery was significantly reduced in chronic smokers (n = 10), hypercholesterolaemic patients (n = 10), and hypertensive patients (n = 8) compared with the controls (n = 10). ◦, +, ‡, $p \leq 0.03$, respectively.

cold pressor testing were reduced compared with patients with normal perfusion imaging (0.75 (0.16) to 0.78 (0.20) ml/g/min *v* 0.75 (0.15) to 1.38 (0.26) ml/g/min, respectively; $p \leq 0.03$). Table 3 shows the results of mean RMBF at baseline, during cold pressor testing, and after sublingual glyceryl trinitrate in the different groups, classified according to their coronary risk factors.

The individual responses of RMBF to cold pressor testing are shown in fig 5 for each group. Non-invasive measurements of RMBF with dynamic PET during cold pressor testing in patients with exercise induced regional myocardial perfusion defects showed a significantly reduced increase in RMBF compared with the control group with normal perfusion images. In the smokers, hypercholesterolaemic patients, and hypertensive patients with exercise induced regional myocardial perfusion defects, the increase in RMBF during cold pressor testing was reduced compared with control group with normal perfusion images: 0.83 (0.10) to 0.78 (0.16) ml/g/min (smoking group), 0.70 (0.18) to 0.80 (0.26) ml/g/min (hypercholesterolaemic group), and 0.72 (0.16) to 0.90 (0.21) ml/g/min (hypertensive group) *v* 0.75 (0.15) to 1.38 (0.26) ml/g/min (control group); $p \leq 0.001$ by analysis of variance (ANOVA) (fig 1).

In group comparisons there were significant differences in RMBF responses to cold pressor testing between patients with exercise induced perfusion defects and those with normal perfusion images ($p \leq 0.03$). Endothelium independent increases in RMBF induced by glyceryl trinitrate did not differ between patients with exercise induced perfusion defects and those with normal perfusion images (0.75 (0.16) to 0.94 (0.09) ml/g/min *v* 0.75 (0.15) to 0.94 (0.09) ml/g/min; NS) (table 3).

Vascular responses of mean myocardial blood flow to cold pressor test

In patients with abnormal regional scintigraphic myocardial perfusion, mean myocardial blood flow responses to cold pressor testing were reduced compared with those with normal perfusion imaging, at 0.75 (0.10) to 0.87 (0.12) ml/g/min *v* 0.76 (0.22) to 1.34 (0.37) ml/g/min ($p \leq 0.03$). In the smokers, hypercholesterolaemic patients, and hypertensive patients, the myocardial blood flow responses to cold pressor testing were impaired compared with the controls: 0.83 (0.05) to 0.79 (0.05) ml/g/min (smoking group), 0.69 (0.15) to 0.90 (0.22) ml/g/min (hypercholesterolaemic group), 0.72 (0.10) to 0.94 (0.10) ml/g/min (hypertensive group) *v* 0.76 (0.22) to 1.34 (0.37) ml/g/min (control group); $p \leq 0.001$ by ANOVA). In group comparisons there were significant differences in mean myocardial blood flow responses to cold pressor testing between patients with exercise induced perfusion defects and those with normal perfusion images ($p \leq 0.03$).

However, in patients with abnormal regional scintigraphic myocardial perfusion, the RMBF response to cold pressor testing was more severely impaired than the mean myocardial blood flow values, though the difference did not reach significance (0.75 (0.16) to 0.78 (0.20) ml/g/min *v* 0.75 (0.10) to 0.87 (0.12) ml/g/min; NS).

Correlation between responses to cold pressor testing of RMBF and exercise induced regional scintigraphic myocardial perfusion defects

RMBF responses to cold pressor testing and the severity of exercise induced scintigraphic regional myocardial perfusion

Table 3 Mean regional myocardial blood flow responses to cold pressor testing and glyceryl trinitrate in patients with regional myocardial perfusion defects and in patients with normal myocardial perfusion (controls)

Group	Baseline	CPT	GTN
Smokers	0.83 (0.10)	0.78 (0.16)*	0.94 (0.05)
Hypercholesterolaemic	0.70 (0.18)	0.80 (0.26)*	0.94 (0.18)
Hypertensive	0.72 (0.16)	0.90 (0.21)*	0.93 (0.05)
Controls	0.75 (0.15)	1.38 (0.26)*	0.94 (0.09)

Values are mean (SD), ml/g/min.
*$p \leq 0.05$ *v* corresponding baseline.
CPT, cold pressor test; GTN, glyceryl trinitrate.

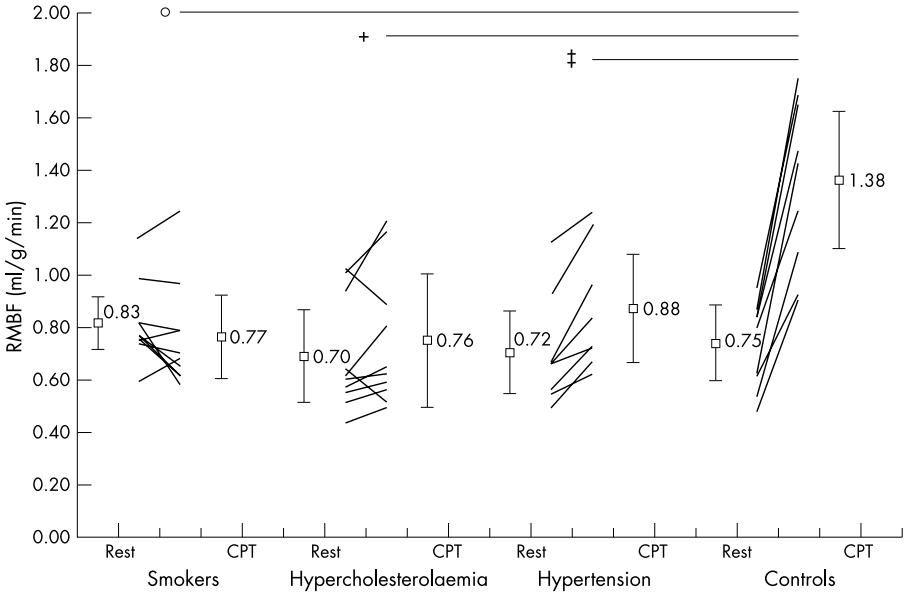

Figure 5 Individual responses of cold pressor test (CPT) induced changes in mean regional myocardial blood flow (RMBF) from baseline in four groups of patients. Significant abnormal endothelium dependent vasomotion is shown in chronic smokers (n = 10), hypercholesterolaemic patients (n = 10), and hypertensive patients (n = 8) compared with controls (n = 10). ◦, +, ‡, $p \leq 0.03$, respectively).

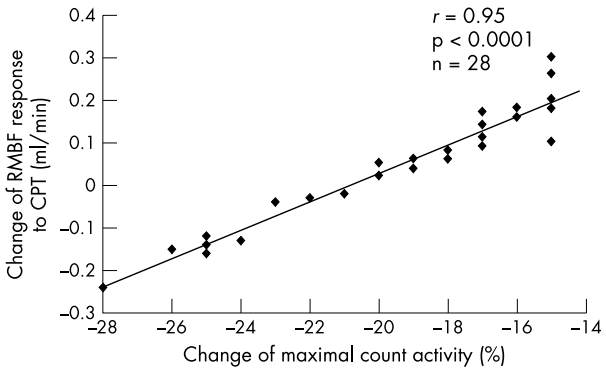

Figure 6 Relation between responses of regional myocardial blood flow (RMBF) to cold pressor testing (CPT) and the severity of exercise induced scintigraphic regional myocardial perfusion defects.

defects were highly correlated ($r = 0.95$, $p = 0.001$), suggesting that abnormal endothelium dependent vasoreactivity of the coronary microcirculation was responsible for the corresponding exercise induced regional myocardial perfusion defects (fig 6). In addition, regression analysis in these patients between RMBF responses to cold pressor testing of the main three vascular territories (left anterior descending, left circumflex, and right coronary arteries) and the severity of the corresponding scintigraphic regional myocardial perfusion defects (22 in the left anterior descending, 13 in the left circumflex, and 14 in the right coronary artery territory) showed significant correlations (left anterior descending: $r = 0.95$, $p = 0.0001$; left circumflex: $r = 0.95$, $p = 0.0001$; right coronary: $r = 0.87$, $p = 0.001$).

DISCUSSION

The major finding in our study is that exercise induced scintigraphic regional myocardial perfusion defects in patients with angina pectoris and patent coronary angiograms may be related to abnormal endothelium dependent vasoreactivity in the corresponding myocardial territory in response to cold pressor testing.

Cold pressor testing has been shown to induce sympathetic stimulation with mixed adrenergic receptor activation of the endothelium and smooth muscle cells of the vascular wall, leading to vasoconstrictor and vasodilator responses; the vasodilator response is thought to be mediated through β adrenoreceptor stimulation and, importantly, through a flow dependent release of nitric oxide (NO) from the endothelium.[6 10 11 18] Accordingly, it has been realised that cold pressor testing represents a non-invasive probe of endothelium dependent coronary vasomotion.[21] It is of note that increased α adrenoreceptor activation in the presence of coronary endothelial dysfunction and atherosclerosis leads to augmented α adrenergic coronary vasoconstriction[22 23]—involving both α1 and α2 adrenoceptors—in the epicardial arteries and microvessels. Hence impairment of the functional integrity of the vascular endothelium in patients with coronary risk factors or established coronary artery disease predisposes the human coronary circulation to enhanced α adrenergic vasoconstriction during sympathetic activation, and may be associated with exercise induced myocardial ischaemia in patients with normal coronary angiography.[12]

However, for decades it has been held that the severity of epicardial coronary artery stenosis correlates with the impairment in coronary flow reserve and with the incidence of myocardial ischaemia.[24] This concept has been widely accepted as the cause of myocardial ischaemia in patients with obstructive coronary artery disease, by not allowing an adequate increase in blood flow with increased metabolic demand.[24] Accordingly, vasodilatation of the coronary resistance vessels can be found in ischaemic myocardial regions. However, there is no simple relation between myocardial blood flow and the severity of coronary artery stenosis.[25]

Experimental and clinical findings have shown active vasoconstriction of coronary vessels during stress testing in myocardial regions with reduced flow and regional hypoperfusion.[26–28] Interestingly, in the presence of significant obstructive coronary lesions the corresponding myocardial perfusion area may have preserved responsiveness to adenosine.[28 29] Several explanations for this phenomenon have been proposed, such as a primary microvascular response to reduced perfusion pressure,[30] active downregulation of myocardial metabolism,[31] or a possible adrenergic activation, as shown in an animal model.[32] However, multiple factors are involved in the regulation of myocardial blood flow, including metabolic and neurohumoral stimuli and physical stimuli such as changes in intraluminal pressure or flow mediated shear stress.[33–35] Following recent investigations, a new concept has been proposed that endothelial dysfunction of the coronary microcirculation contributes to myocardial perfusion defects.[5 12]

In this study we showed that in patients with exercise induced scintigraphic regional myocardial perfusion defects,

abnormal endothelium dependent vasoreactivity of the epicardial coronary arteries extended into the coronary microcirculation, whereas patients with normal perfusion images had normal endothelium dependent vasoreactivity. The responses of both epicardial arteries and coronary blood flow to cold pressor testing in patients with exercise induced myocardial perfusion defects were significantly impaired compared with those in patients with normal homogeneous myocardial perfusion. Interestingly, the degree of abnormal endothelium dependent vasoreactivity in the coronary circulation was more severe in chronic smokers than in hypercholesterolaemic or hypertensive patients, emphasising the deleterious effects of cigarette smoking on the vascular endothelium.[6] In line with previous findings,[11 12] resting myocardial blood flow in hypertensive patients with exercise induced regional myocardial perfusion defects did not differ from that in patients with normal scintigraphic myocardial perfusion. In our study, all the patients had normal left ventricular mass indices as assessed by echocardiography; hence any potentially confounding effects of left ventricular hypertrophy on coronary blood flow can be excluded. This is in agreement with previous results from Quyymi and colleagues,[36] showing a lack of effect of hypertension on coronary blood flow responses to atrial pacing and acetylcholine in patients with microvascular angina. However, assessment of glyceryl trinitrate induced, endothelium independent vasodilatation of the coronary circulation showed no significant differences between subgroups of patients with and without coronary risk factors and exercise induced regional myocardial perfusion defects. This ruled out altered responsiveness of the smooth muscle cells as a cause of the scintigraphic perfusion defects. It is noteworthy that although exogenous nitric oxide given in glyceryl trinitrate induces an endothelium independent myocardial blood flow response, it has only minor vasodilator effects on coronary resistance vessels—as shown in the present study—and, importantly, it cannot substitute for the central role of nitric oxide release from the vascular endothelium in the regulation of myocardial blood flow during times of increased metabolic demand.[6 37 38]

On the basis of the presumed function of the vascular endothelium in maintaining myocardial blood flow during increased metabolic demand,[6–9] dysfunctional endothelium may be an important determinant of exercise induced myocardial perfusion defects in patients with normal coronary angiograms. Indeed, there was a highly significant correlation between the impairment of endothelium dependent regional myocardial blood flow responses to cold pressor testing and the severity of exercise induced scintigraphic perfusion defects, strongly suggesting abnormal endothelium dependent vasoreactivity of the corresponding myocardial territory as a cause for such myocardial perfusion defects. In this regard, previous clinical studies have provided the first evidence that endothelial dysfunction of coronary arteriolar vessels assessed by acetylcholine may be associated with scintigraphic myocardial perfusion defects of the anterior wall before intracoronary infusion of the highest doses of acetylcholine[5] or during increased metabolic demand.[12]

Although the role of acetylcholine in determining endothelial function in the coronary circulation has certainly been appreciated, it is not likely to be an important physiological regulator of vasomotor tone. In contrast, a more physiological stimulus such as cold pressor testing[10 18] to determine endothelium dependent coronary vasoreactivity may be difficult to interpret in view of simultaneous changes in multiple variables such as activation of the sympathetic nervous system, augmentation of coronary blood flow, and an increase in myocardial workload, but it is of great relevance in relating endothelial dysfunction to myocardial ischaemia during daily life[39] and during exercise.[10 40] Buchthal and colleagues reported the first direct evidence of an abnormal metabolic response to stress testing in 20% of woman with chest pain in the absence of angiographically significant coronary artery stenoses, as assessed by phosphorus-31 nuclear magnetic resonance spectroscopy (^{31}P-NMR).[41]

A limitation of the latter investigations[5 12 41] was that the assessment of abnormal endothelium dependent vasomotor or metabolic responses were confined to the left anterior wall. To this end, we applied three dimensional scintigraphic fusion imaging[13–15] for accurate assignment of exercise induced regional myocardial perfusion defects to the corresponding epicardial vessel segment. In line with previous reports,[5 12] our three dimensional images showed that 45% of the perfusion defects were in the myocardial territory supplied by the left anterior descending coronary artery. However, perfusion defects were not confined to the anterior wall but were also observed in the remaining left ventricular territories: those supplied by the right coronary artery in 21%, by the left circumflex coronary artery in 18%, and by the posterolateral and obtuse marginal branches in 8%. Hence, abnormal endothelium dependent vasomotion of myocardial territories following cold pressor testing was associated with corresponding exercise induced myocardial perfusion defects, probably reflecting spatial differences in the endothelium dependent increases in regional myocardial blood flow.[42] It is noteworthy that, while the impairment of RMBF in response to cold pressor testing in regions with scintigraphic perfusion defects was more severe than the impairment of mean myocardial blood flow in the remaining vascular territories, the difference was not significant, indicating that abnormal endothelium dependent vasoreactivity was not restricted to the myocardial area of reduced scintigraphic perfusion during exercise.

Exercise induced myocardial perfusion defects were assessed by thallium perfusion imaging rather than by ECG.[43] A common misconception is that myocardial ischaemia must be induced to cause perfusion defects. Myocardial ischaemia or hypoxia decreases nuclear tracer uptake only slightly; thus the predominant reason for a stress induced perfusion defect is the differential increase in coronary blood flow.[44] This may explain why exercise induced perfusion defects are much more often observed than ECG abnormalities or angina during stress. Furthermore, findings compatible with myocardial ischaemia (for example, stress induced scintigraphic myocardial perfusion defects, left ventricular dysfunction on exercise, and myocardial lactate production) have been observed in patients who fail to develop ST segment depression during exercise testing.[43 45–47] In this study, significant ST segment depression during exercise was induced in 71% of cases in patients with scintigraphic regional myocardial perfusion defects and in 25% of cases in patients with normal myocardial perfusion. We reasoned that exercise induced ST segment depression in patients with normal coronary angiograms and normal scintigraphic myocardial perfusion may be attributed to neurohumoral "electrophysiological" causes rather than to ischaemia.[48] Indeed, it is likely that exercise induced ST segment depression may be related to both non-ischaemic and ischaemic causes.[49] However, we cannot deduce the exact mechanisms underlying ST segment depression during exercise from our data. To complicate matters, Lanza and colleagues reported patients with anginal chest pain, normal coronary arteries, and a normal ECG during stress testing who developed episodes of ischaemia-like ST segment depression during daily life as assessed by Holter monitoring,[39] suggesting that vasomotor mechanisms influencing myocardial blood flow may be more active than, or different from, those in patients with coronary artery disease.[50] This is in line with circadian variation in coronary vasomotor tone or endothelial function, resulting in temporal variations in myocardial perfusion during daily life or exercise testing.[39 51 52]

Coronary microvascular dysfunction with evidence of myocardial ischaemia may be representative of the so called syndrome X,[2] which is characterised by chest pain and

regional ischaemia associated with an overall reduction in coronary flow reserve, but without depression of left ventricular performance. Several groups[5 12 13] have suggested that endothelial dysfunction may be involved in the pathophysiology of syndrome X by causing exercise induced vasoconstriction of arteriolar vessels. As typical chest pain is not observed in all patients with endothelial dysfunction of the coronary microcirculation,[6] it seems unlikely that endothelial dysfunction always accounts for the clinical symptoms in patients with syndrome X. More probably, endothelial dysfunction is associated with an abnormal cardiac pain perception causing angina in these patients.[53]

Limitations

There are several limitations to the interpretation of our data. The results presented were obtained from a sample of highly selected patients referred for coronary angiography to evaluate persistent chest pain. Hence the proportion of patients with endothelial dysfunction revealed by cold pressor testing associated with myocardial perfusion defects during exercise may be different among patients examined for chest pain in other settings.

A further limitation of our study is that we could not undertake coronary angiography during exercise to demonstrate the extent of exercise induced coronary epicardial artery constriction. It is conceivable that exercise might have induced significant epicardial artery constriction, limiting coronary blood flow in patients with exercise induced myocardial perfusion defects. In addition, although patients with evidence of myocardial bridging of coronary vessels during coronary angiography were not included in the study, we cannot exclude the possibility that this form of coronary anomaly may have become apparent during exercise, contributing to regional myocardial perfusion defects. However, no patient had angiographically significant coronary artery narrowing or ST segment elevation typical of the presence of variant angina, indicating that impaired coronary blood flow during exercise may not have resulted from excessive vasoconstriction of the epicardial coronary artery.[54]

One further important drawback was that we did not undertake intravascular ultrasound to assess the vascular wall structure for an accurate classification of patients with normal coronary angiograms.[55] As reported previously,[56] early signs of atherosclerosis not detected by coronary angiography are often encountered in patients with angina. Hence in the present study, despite normal coronary angiography, diffuse atherosclerosis may have been present; this has been reported to be associated with longitudinal, base to apex myocardial perfusion abnormalities[57] and with augmented α adrenergic coronary vasoconstriction.[22 23]

Finally, although in our study abnormal endothelium dependent vasoreactivity of the coronary circulation in response to cold pressor testing was associated with a heterogeneous exercise induced distribution of myocardial perfusion defects, this does not imply that endothelial dysfunction is the only underlying mechanism. As we did not assess myocardial blood flow reserve in response to adenosine,[58] we do not know whether exercise induced myocardial perfusion defects were secondary to impaired augmentation of coronary blood flow resulting in a reduced flow dependent dilatation, or whether they were primarily suggestive of a more severely reduced vasodilator capacity. In this regard, determination of myocardial blood flow reserve is desirable in patients with exercise induced regional myocardial scintigraphic defects and abnormal endothelium dependent vasoreactivity of the coronary circulation.

Clinical implications

Regardless of the exact mechanism responsible for abnormal endothelium dependent vasomotion of the coronary circulation, we showed the presence of exercise induced scintigraphic regional myocardial perfusion defects in patients with angina but normal coronary angiography. We were able to relate this observation to abnormal blood flow mediated vasoreactivity of the corresponding myocardial territory in response to cold pressor testing. These observations support the clinical relevance of endothelium dependent vasomotor tone in producing regional myocardial perfusion defects.

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