Tetrahydrobiopterin restores impaired coronary microvascular dysfunction in hypercholesterolaemia

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Abstract. *Purpose:* Tetrahydrobiopterin (BH_4) is an essential co-factor for the synthesis of nitric oxide (NO), and BH_4 deficiency may cause impaired NO synthase (NOS) activity. We studied whether BH_4 deficiency contributes to the coronary microcirculatory dysfunction observed in patients with hypercholesterolaemia.
Methods: Myocardial blood flow (MBF; ml min^{-1} g^{-1}) was measured at rest, during adenosine-induced (140 μg kg^{-1} min^{-1} over 7 min) hyperaemia (mainly non-endothelium dependent) and immediately after supine bicycle exercise (endothelium-dependent) stress in ten healthy volunteers and in nine hypercholesterolaemic subjects using ^{15}O-labelled water and positron emission tomography. Measurements were repeated 60 min later, after intravenous infusion of BH_4 (10 mg kg^{-1} body weight over 30 min). Adenosine-induced hyperaemic MBF is considered to represent (near) maximal flow. Flow reserve utilisation was calculated as the ratio of exercise-induced to adenosine-induced hyperaemic MBF and expressed as percent to indicate how much of the maximal (adenosine-induced) hyperaemia can be achieved by bicycle stress.
Results: BH_4 increased exercise-induced hyperaemia in controls (2.96±0.58 vs 3.41±0.73 ml min^{-1} g^{-1}, $p<0.05$) and hypercholesterolaemic subjects (2.47±0.78 vs 2.70±0.72 ml min^{-1} g^{-1}, $p<0.01$) but had no influence on MBF at rest or during adenosine-induced hyperaemia in controls (4.52±1.10 vs 4.85±0.45 ml min^{-1} g^{-1}, p=NS) or hypercholesterolaemic subjects (4.86±1.18 vs 4.53±0.93 ml min^{-1} g^{-1}, p=NS). Flow reserve utilisation remained unchanged in controls (70±17% vs 71±19%, p=NS) but increased significantly in hypercholesterolaemic subjects (53±15% vs 66±14%, $p<0.05$).
Conclusion: BH_4 restores flow reserve utilisation of the coronary microcirculation in hypercholesterolaemic subjects, suggesting that BH_4 deficiency may contribute to coronary microcirculatory dysfunction in hypercholesterolaemia.

Keywords: Nitric oxide synthase – Endothelium – Coronary circulation – PET – Bicycle exercise

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Introduction

In hypercholesterolaemic patients, endothelial-mediated dilation of angiographically normal coronary arteries is impaired [1, 2]. This endothelial dysfunction is, at least in part, reversible by L-arginine infusion and treatment with lipid-lowering drugs or can be compensated by the effect of calcium channel blockers on vascular smooth muscle cells [3]. From in vitro results, endothelial dysfunction has been attributed to attenuation of nitric oxide (NO) release and/or superoxide anion production induced by oxidised low-density lipoprotein (LDL) cholesterol. In studies with positron emission tomography (PET), a reduction in coronary flow reserve (CFR) has been documented in hypercholesterolaemic asymptomatic subjects with normal coronary arteries and mainly associated with the LDL lipid fraction rather than with total cholesterol levels [4]. CFR and flow-mediated dilation are impaired owing to reduced NO production or release [5, 6].

NO synthase (NOS) produces both NO and, under certain conditions, superoxide anion ($O^{\bullet}$). A physiologi-

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cal balance between NO and $O^{\bullet}$ requires an adequate concentration of tetrahydrobiopterin (BH_4), an essential co-factor of the NOS complex. Decreased BH_4 concentration in endothelial cells of patients with hypercholesterolaemia has been associated with increased $O^{\bullet}$ production and endothelial dysfunction [7]. Furthermore, improvement of coronary endothelial function has been demonstrated after intracoronary infusion of BH_4 in hypercholesterolaemic patients [8]. It remains unclear, however, whether these phenomena extend to the coronary microcirculation and, thus, to the regulation of myocardial blood flow (MBF).

The aim of this study was to examine whether systemic BH_4 administration restores endothelial-dependent regulatory mechanisms of MBF in asymptomatic subjects with hypercholesterolaemia. To stimulate endothelial NOS (eNOS) in a shear stress-dependent manner, bicycle exercise was used, while adenosine, which exerts its effect largely independently of NO, served as control.

Materials and methods

Study population

Ten healthy males (mean age 25±3 years) and nine subjects with hypercholesterolaemia (mean age 54±8 years) were included in the study. The baseline characteristics are summarised in Table 1. Both groups had no history of and low clinical probability for coronary artery disease. Entry criteria included normal heart rate, blood pressure and electrocardiogram (ECG). None of the subjects had any cardiovascular risk factors except for hypercholesterolaemia in the hypercholesterolaemic group, and all subjects were free of cardiovascular symptoms. Lipid profile was normal in all volunteers (total cholesterol 4.4±0.4 mmol l^{-1}, ratio of total to HDL cholesterol 3.3±0.5) and abnormal in the hypercholesterolaemic group according to the inclusion criteria (total cholesterol 8.7±2.1 mmol l^{-1}, ratio of total to HDL cholesterol 6.7±2.9). All subjects refrained from ingesting caffeinated beverages or food for 24 h before the study. None of the hypercholesterolaemic subjects had been on statins for at least 6–8 weeks.

Table 1. Baseline patient characteristics

	Controls	Hypercholesterolaemics	*p*
No.	10	9	
Age	25±3 years	54±8 years	<0.0001
Gender (m/f)	10/0	7/2	
Cholesterol			
Total cholesterol	4.4±0.4	8.7±2.1	<0.0001
HDL	1.4±0.3	1.4±0.3	NS
Ratio (total chol./HDL)	3.2±0.5	6.7±2.9	<0.005
LDL	2.6±0.5	6.0±1.7	<0.0001
Triglycerides	0.9±0.3	2.8±1.5	<0.005

Study protocol

With the subject's feet attached to a bicycle ergometer (model 380 B, Siemens-Elema AG, Switzerland), MBF was measured at rest and repeated during adenosine-induced hyperaemia after a 10-min period to allow for decay of ^{15}O radioactivity. Adenosine was infused for 7 min at 140 μg per kg body weight per minute according to standard practice. Three minutes after the start of the adenosine infusion, the hyperaemic MBF measurement was started. After a 10-min interval, exercise was started at 50–75 W, and workload was increased in increments of 25 W min^{-1} to reach 70% of the predicted value for upright bicycle exercise within a comparable time period in all subjects. MBF measurement was performed immediately after the end of exercise as recently documented [9, 10]. This was followed by an intravenous BH_4 infusion (10 mg kg^{-1}) over 30 min and by an additional break of 30 min to allow for the maximal vasodilating effect of BH_4 [11]. During this time, a 20-min transmission scan was acquired for the purpose of attenuation correction of all emission scans. Sixty minutes after starting the BH_4 infusion, a repeat series of three MBF measurements was performed using the same protocol as was employed at baseline. The BH_4 dose of 10 mg kg^{-1} was chosen according to previous dose-finding studies in which this dose had proved safe and effective in the coronary circulation of healthy volunteers [11].

Blood pressure was continuously monitored by a Finapress BP Monitor (BOC, Inc, Englewood, CO, USA) and recorded at baseline and every minute during adenosine administration as well as at each exercise level and during 10 min of recovery. The ECG was monitored continuously throughout the procedure and a 12-lead ECG was recorded at identical times as blood pressure.

Repeatability substudy

In an additional subgroup of five hypercholesterolaemic subjects (total cholesterol 7.0±0.9 mmol l^{-1}, ratio of total to HDL cholesterol 6.34±0.74), saline was infused instead of BH_4 to assess the repeatability of the measurements.

Image acquisition

MBF was assessed in the PET Center of the University Hospital in Zurich on a GE advance positron emission tomograph (GE Medical Systems, Milwaukee, WI, USA). Starting after the background frame, ^{15}O-water (500–700 MBq) was injected as an intravenous bolus over 20 s at an infusion rate of 10 ml min^{-1} to assess MBF. The line was then flushed for another 2 min. The dynamic image sequences were: 14×5 s, 3×10 s, 3×20 s and 4×30 s.

Image processing

The obtained sinograms were corrected for attenuation and reconstructed on a SUN workstation (Sun Microsystems, Mountain View, CA) using standard reconstruction algorithms. Images were transferred to a transtec 2200 PC (transtec Computer AG, Bulach, Switzerland) and analysed with the PMOD (PMOD Technologies GmbH, www.pmod.ch) software package designed and validated at our institution [9, 12]. Myocardial images were generated directly from the dynamic ^{15}O-water study by means of linear di-

mension reduction of the dynamic sinograms as previously described [13]. Regions of interest were drawn within the left ventricular cavity and myocardium on consecutive image planes and projected onto the dynamic ^{15}O-water images to generate blood time-activity curves. These curves were fitted to a single tissue compartment tracer kinetic model to give values of regional and global MBF (ml min^{-1} g^{-1}) [4, 13, 14]. To account for the variability of coronary driving pressure, coronary resistance (mmHg ml^{-1} min^{-1} g^{-1}) was also calculated as the ratio of mean arterial pressure to MBF at rest, during adenosine infusion and after bicycle stress [10, 15].

Flow reserve utilisation

Adenosine-induced hyperaemic MBF is considered to represent (near) maximal flow. Flow reserve utilisation was calculated for each subject as the ratio of exercise-induced to adenosine-induced hyperaemic MBF and expressed as percent to indicate how much of the maximal (adenosine-induced) hyperaemia can by achieved by bicycle exercise. This index is comparable in different circumstances if external cardiac workload (rate–pressure product, RPP) remains the same. Flow reserve utilisation represented the primary endpoint of the study. A concept of flow reserve utilisation has been previously proposed by Zeiher et al. [16]. In their study, however, they assessed the capacity of the coronary system to increase MBF in response to acetylcholine. Thus, the acetylcholine dose–response relation was expressed as relative proportion of the maximally obtainable coronary MBF response to papaverine.

Preparation of tetrahydrobiopterin (BH_4)

A sodium bicarbonate-buffered solution of 10 mg kg^{-1} of (6*R*)-5,6,7,8-tetrahydro-L-biopterin-dihydrochloride (BH_4, Dr B. Schircks Laboratories, Jona, Switzerland) was prepared as previously described [11] in a total volume of 10 ml immediately before use and diluted with 0.9% sodium chloride to a total volume of 50 ml. The sterilely filtered, clear, colourless solution was infused into a peripheral vein of the forearm using a pump at a constant flow of 99.9 ml h^{-1}.

Statistical analysis

Data are reported as mean values ± standard deviation (SD) if not otherwise stated. Intergroup comparisons of haemodynamic and PET data at rest and during both stress modalities were carried out by a two-way analysis of variance (ANOVA) for repeated measurements, followed by the Scheffé *F* test when the ANOVA test was significant ($p<0.05$).

Results

None of our subjects experienced adverse effects from adenosine.

Haemodynamics

Resting heart rate (beats per minute) was slightly higher after BH_4 infusion in hypercholesterolaemic subjects (65±10 vs 70±9, $p<0.005$) whereas it was unchanged in control subjects (68±10 vs 70±9, NS). After BH_4 infusion, diastolic blood pressure (mmHg) increased significantly at rest (68±15 vs 76±13, $p<0.05$) and during adenosine (66±13 vs 76±9, $p<0.05$) in controls but remained unchanged in hypercholesterolaemic subjects (rest: 64±13 vs 70±11, NS; adenosine: 61±9 vs 67±5, NS). All the other haemodynamic parameters were similar before and after BH_4 for all study conditions in controls and in hypercholesterolaemic subjects, including the post-exercise period (values averaged over 4 min) during which MBF measurement was performed (Table 2). Further-

Table 2. Haemodynamics

	Rest			Adenosine			Exercise[a]		
	Baseline	BH_4	*p*	Baseline	BH_4	*p*	Baseline	BH_4	*p*
Controls (*n*=10)									
SBP	125±11	123±13	NS	133±11	134±11	NS	129±11	135±10	NS
DBP	68±15	76±13	<0.05	66±13	76±9	<0.05	72±9	78±8	NS
MAP	87±13	92±12	NS	89±12	95±7	NS	91±9	97±6	NS
HR	68±10	70±9	NS	93±14	97±16	<0.05	99±15	98±17	NS
RPP	8,483±1,381	8,634±1,901	NS	12,422±2,411	13,039±2,766	NS	12,699±1,967	13,652±2,236	NS
Hypercholesterolaemics (*n*=9)									
SBP	139±16	137±12	NS	142±14	143±10	NS	141±18	147±10	NS
DBP	64±13	70±11	NS	61±9	67±5	NS	66±12	69±11	NS
MAP	89±13	92±11	NS	88±10	92±6	NS	91±14	92±6	NS
HR	65±10	70±9	<0.005	85±15	89±12	NS	86±16	85±15	NS
RPP	9,104±2,225	9,608±1,636	NS	12,052±2,185	11,210±4,437	NS	11,429±1,941	11,623±1,407	NS

SBP systolic blood pressure, *DBP* diastolic blood pressure, *MAP* mean arterial pressure, *HR* heart rate, *RPP* rate–pressure product
[a] Haemodynamic values immediately post exercise (averaged over 4 min).

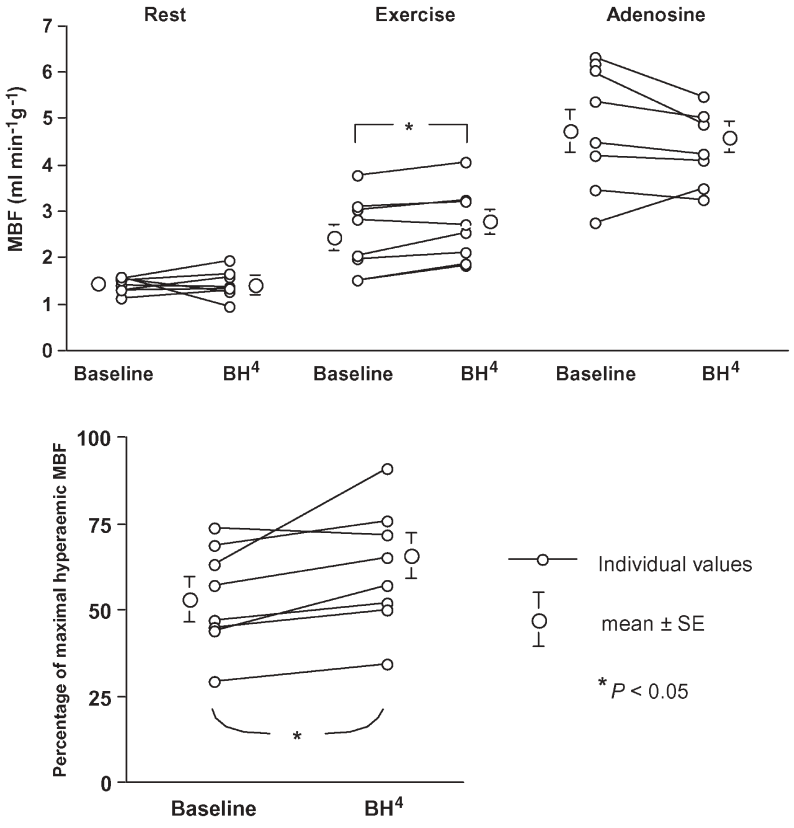

Fig. 1. Individual values of MBF and flow reserve utilisation are given for hypercholesterolaemic subjects before and after BH_4

Table 3. MBF and coronary flow reserve utilisation

	Controls			Hypercholesterolaemics		
	Baseline	BH_4	*p*	Baseline	BH_4	*p*
MBF rest (ml min^{-1} g^{-1})	1.33±0.42	1.38±0.37	NS	1.46±0.18	1.42±0.27	NS
MBF adenosine (ml min^{-1} g^{-1})	4.52±1.10	4.85±0.45	NS	4.86±1.18	4.53±0.93	NS
MBF bicycle (ml min^{-1} g^{-1})	2.96±0.58	3.41±0.73	<0.05	2.47±0.78	2.70±0.72	<0.01
CFR utilisation (%)	70±17	71±19	NS	53±15	66±14	<0.05

more, RPP during maximal bicycle exercise stress was not affected by BH_4 in controls (26,544±4,929 vs 25,197±5,188, NS) or in hypercholesterolaemic subjects (23,774±4,566 vs 24,005±6,424, NS). RPP values did not differ between the two groups, confirming similar workload during exercise and allowing meaningful comparison of MBF between the two groups, particularly in view of age-related differences in exercise performance.

MBF, coronary resistance and flow reserve utilisation

Values for repeat measurements of MBF were 1.16± 0.38 vs 1.15±0.31 ml min^{-1} g^{-1} at rest, 2.92±1.11 vs 2.69±0.69 ml min^{-1} g^{-1} during adenosine, and 2.12± 0.32 vs 1.99±0.45 ml min^{-1} g^{-1} after exercise. The respective mean differences were not significant (–2% for rest, –5% for exercise, –12% for adenosine). Values of mean MBF and CFR utilisation before and after BH_4 are reported in Table 3. Individual values of hypercholesterolaemic subjects are given in Fig. 1. Resting and adenosine-induced hyperaemic MBF were not affected by BH_4 either in controls or in hypercholesterolaemic subjects. By contrast, MBF immediately after exercise was significantly increased by BH_4 infusion both in controls (2.96±0.58 to 3.41±0.73 ml min^{-1} g^{-1}, $p<0.05$) and in hypercholesterolaemic subjects (2.47±0.78 to 2.70± 0.72 ml min^{-1} g^{-1}, $p<0.01$).

Coronary resistance was not affected by BH_4 in either group at rest or during adenosine while it decreased by 15% after bicycle exercise in hypercholesterolaemic subjects (from 44 to 37 mmHg ml^{-1} min^{-1} g^{-1}) but only by 8% in controls (from 31 to 29 mmHg ml^{-1} min^{-1} g^{-1}).

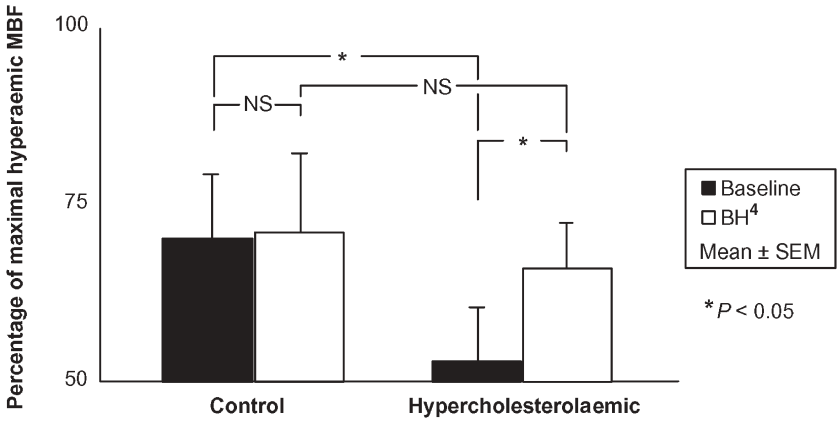

Fig. 2. Flow reserve utilisation at baseline and after BH_4 infusion in controls and hypercholesterolaemics. As RPP was almost identical before and after BH_4, the changes are not due to changes in cardiac work but indicate improved endothelial function

BH_4 had no influence on flow reserve utilisation in controls (70±17% vs 71±19%, p=NS for baseline versus BH_4 infusion). Compared with controls, flow reserve utilisation was significantly lower in hypercholesterolaemic subjects at baseline (53±15%; $p<0.05$ vs controls) and increased significantly after BH_4 infusion (66±14%, $p<0.05$ vs baseline), approximating values similar to controls (p=NS vs controls) (Fig. 2).

Discussion

This study demonstrates that in hypercholesterolaemia, flow reserve utilisation after physical exercise is significantly reduced compared with that in controls but is nearly restored after BH_4 infusion. BH_4 increased microcirculatory response to physical exercise, which is known to activate eNOS, but not to the endothelium-independent vasodilator adenosine. While previous studies have shown a favourable effect of BH_4 on conductance arteries of the peripheral circulation [7] and epicardial arteries [8] of subjects with hypercholesterolaemia, our data extend these findings to the coronary microcirculation and the regulation of MBF. Our data show that CFR utilisation is significantly impaired in hypercholesterolaemia, but restored after BH_4 infusion.

In agreement with our previous results, we found changes in MBF after BH_4 without concomitant haemodynamic changes. Although non-invasive arterial blood pressure monitoring may not be the most sensitive method for detection of subtle peripheral vasodilatation, the present data indicate that BH_4 at the dose of 10 mg kg^{-1} has no peripheral vasodilating effect. This suggests either a lower functional BH_4 concentration in coronary endothelial cells or a greater susceptibility of the coronary circulation to BH_4 as compared with the systemic vascular bed. When defining all subjects with an increase in flow reserve utilisation of 10% or more as responders, none of the healthy volunteers was a responder, while six of the nine hypercholesterolaemic subjects were responders. Among the hypercholesterolaemic subjects, the non-responders tended to have the highest lipid values, potentially indicating that the BH_4 supplementation at the provided dose was insufficient. Due to the limited numbers in this subpopulation, however, such a statement remains speculative.

Several pharmacological agents have been proposed to study the role of the endothelium of normal and stenotic coronary arteries, namely serotonin, noradrenaline, vasopressin, adenosine, papaverine or acetylcholine [17]. In comparison with most previous studies, a different approach was used in the present study to induce coronary vasodilation, namely dynamic bicycle exercise—a powerful predictor of outcome and mortality as recently confirmed in a large long-term trial [18]. The effect of exercise on coronary vasomotion is probably more complex than that of a single pharmacological agent. A pharmacological stimulus alone may have been of limited value in the assessment of the coronary microcirculatory function. However, very few reports in the literature deal with the use of physical exercise in PET [19–21] and only recently has its repeatability been documented in healthy volunteers [9]. In this study, we have provided the repeatability of the measurements for hypercholesterolaemic subjects. The mean differences between the two repeat measurements were 2%, –5% and –12% for rest, exercise and adenosine, respectively, comparing well with the values reported in the literature by us [9, 14] and others [22].

Seiler et al. [23] have reported that exercise-induced endothelium-mediated vasodilation of angiographically smooth coronary arteries is inversely related to serum cholesterol as well as LDL cholesterol levels. The present study is the first to use physical exercise and PET MBF measurement to document the impact of hypercholesterolaemia (and of BH_4 supplementation) on the coronary circulation.

Endothelium-independent and -dependent coronary hyperaemic response

The vasodilator effect of adenosine has been generally reported to be mainly based on the direct stimulation of

$A2_A$ adenosine receptors on vascular smooth cells. Therefore, adenosine has frequently been used to evaluate endothelium-independent vasodilation [24]. Recently, it has been appreciated that adenosine acts in part as an endothelium-dependent vasodilator, although a large proportion of its action is endothelium independent [6]. We used adenosine as a stimulus because it induces (near) maximal hyperaemia and, thus, allows comparison of exercise-induced hyperaemia with the maximal MBF achievable in each individual subject. This strategy was chosen to allow differentiation between endothelial-dependent (bicycle exercise) and predominantly endothelial-independent (adenosine) hyperaemia. The flow reserve utilisation indicates how much of the maximal available hyperaemic MBF can be utilised during physical exercise.

Exercise stress is a complex stimulus of MBF, but probably more comparable to daily physical activities [25–28] than other stimuli such as pharmacological vasodilators or pacing stress. Both intracoronary acetylcholine [29] and dynamic exercise [3, 25, 28] have been shown to constrict the coronary arteries in the presence of an atherosclerotic lesion but to dilate normal coronary vessels. Thus, physical exercise seems to be a reliable tool for testing coronary endothelial function. In particular, physical exercise stimulates eNOS activity in a shear stress-dependent manner [27, 30, 31]. In conjunction with PET this provides a new non-invasive tool for assessing physiological mechanisms of coronary vasomotor control, which regulate absolute quantitative MBF.

Exercise-induced hyperaemic MBF may result not only from an increase in oxygen demand producing more metabolic vasodilation, but also from either direct stimulation of α_2-adrenoceptors in intact endothelial cells and the release of NO [32] or direct β_2-adrenoceptor-mediated dilation of coronary arterioles by noradrenaline [33]. Endothelial integrity seems to play a major role in exercise-induced hyperaemia as early stages of endothelial dysfunction and atherosclerosis already impair coronary dilator responses. In fact, in angiographically normal epicardial vessels, exercise-induced vasodilation is attenuated or completely blunted in the presence of hypertension or hypercholesterolaemia [3, 28, 30, 34].

Both stimuli, i.e. adenosine and bicycle stress, induce a hyperaemic MBF response mediated via a complex combination of sympathetic efferents [15], adenosine receptor activation and other mechanisms. However, exercise-induced hyperaemic MBF is predominantly endothelium dependent [3, 28, 30, 34] whereas adenosine-induced hyperaemic MBF is predominantly endothelium independent [24]. In order to cancel out the non-endothelial-dependent mechanisms and to most selectively assess endothelial function, we therefore expressed the exercise-induced hyperaemic MBF in relation to the adenosine-induced response, proposing (percent) flow reserve utilisation as an index of endothelial function.

The observed increase in exercise MBF in healthy volunteers could be due to a direct unspecific anti-oxidant effect of BH_4 similar to that of vitamin C, which has been shown to improve endothelial function in healthy volunteers by protecting NO from inactivation by oxygen free radicals. Alternatively, BH_4 may have improved endothelial-dependent vasodilation specifically through the increased production of NO. This confirms a recent report on further improvement of endothelial function by aerobic exercise in healthy volunteers who do not have endothelial dysfunction [35].

BH_4 and flow reserve utilisation

Flow reserve utilisation was significantly lower in hypercholesterolaemic than in control subjects but was restored after BH_4 infusion. Under high-flow conditions such as bicycle exercise stress, BH_4 supplementation seems to have a favourable effect by increasing the exercise-induced flow response, possibly by counteracting short-term relative substrate depletion. Alternatively, reduced NO bioavailability may reflect increased breakdown by superoxide anions. NOS itself may be a source of superoxide anion production at suboptimal concentrations of BH_4.

Study limitations

Data acquisition was obtained in the immediate post-exercise period when the cardiac power output is considerably decreased and when flow is expected to fall rapidly. These suboptimal conditions were chosen to avoid excessive motion artefact during scanning [9, 10]. As a consequence, we could not assess maximal flow during peak exercise but rather assessed the average flow during the first minutes of recovery. This seems a potential limitation of our study as the maximal impact of BH_4 is anticipated during high-flow conditions with maximal shear stress such as peak flow. Nevertheless, we found that BH_4 induced a significant improvement in microcirculatory dysfunction under submaximal flow conditions, which strengthens our result. The present protocol provides the response to physical exercise, which is reproducible [9] and is appropriate for study of the influence of interventions on exercise-induced (endothelium-dependent) CFR [10].

Hypercholesterolaemic subjects were older than controls. This difference may potentially have hampered the comparability of the two groups' flow reserve as the flow reserve has been shown to decrease after the age of 60 [36], though mainly due to an increase in basal flow [37]. By contrast, however, maximal hyperaemic response decreases only after the age of 70 years [38], and this age-related effect is irrelevant to our study as none of our patients was older than 70 years. The use of every subject as his or her own control further strengthens our results, although theoretically it cannot be entirely ex-

cluded that at different ages BH_4 may exert differential unspecific anti-oxidant effects.

RPP was almost identical before and after BH_4 under the different conditions, indicating that the improvement in flow reserve utilisation was not due to changes in cardiac work but reflected improved endothelial function in hypercholesterolaemic subjects at a workload targeted to 70% of the predicted workload according to the study protocol.

As none of the subjects underwent coronary angiography, subclinical CAD cannot be ruled out with certainty. However, the clinical risk for coronary artery disease was assessed as low before enrolment. In addition, it has recently been demonstrated that even in patients with mild CAD, flow assessed with PET can still be used to evaluate the functional response of the coronary artery circulation [2].

Conclusion

Acute administration of BH_4 seems to restore CFR utilisation in hypercholesterolaemic subjects, suggesting that BH_4 deficiency may contribute to coronary microcirculatory dysfunction in hypercholesterolaemia.

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