Metabolic vasodilation in the human forearm is preserved in hypercholesterolemia despite impairment of endothelium-dependent and independent vasodilation

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Abstract

Objective: Hypercholesterolemia has been shown to impair endothelium-mediated, nitric oxide (NO)-dependent responses to acetylcholine (ACh), serotonin, substance P and flow-mediated dilation. We have recently shown that NO contributes to metabolic vasodilation in the human forearm. We sought to determine whether metabolic vasodilation is impaired in healthy subjects with hypercholesterolemia.

Methods: We compared the forearm blood flow (FBF) responses to isotonic exercise, ACh and the endothelium-independent vasodilator sodium nitroprusside in young, otherwise healthy volunteers with hypercholesterolemia and controls before and after the NO inhibitor N\textsuperscript{G}-monomethyl-L-arginine (L-NMMA). FBF was measured using venous occlusion plethysmography. Hypercholesterolemic (n=20) and control (n=20) subjects were age- and gender-matched.

Results: Total cholesterol (6.9±0.3 vs. 4.6±0.1 mmol/l, P<0.0001), low density lipoprotein (4.9±0.4 vs. 2.7±0.1 mmol/l, P<0.001) and triglyceride (1.3±0.2 vs. 0.8±0.1 mmol/l, P=0.005) levels were higher in the hypercholesterolemic group. Basal FBF and resistance were similar in the two groups. Hypercholesterolemia impaired the peak FBF response to ACh (11.1±1.9 vs. 17.6±2.2 ml/100 ml/min, P=0.03), and reduced the peak response to sodium nitroprusside (6.0±0.4 vs. 8.1±0.6 ml/100 ml/min, P<0.01). However, hypercholesterolemia did not affect peak hyperemic FBF (13.1±1.0 vs. 13.2±1.0 ml/100 ml/min, P=1.0) or the FBF volume repayment during the 1 or 5 min after exercise. Resting FBF was reduced by L-NMMA to a similar degree (by 33% vs. 40%, P=0.17) in both groups. Although L-NMMA reduced peak hyperemic FBF (by 16% vs. 17%, P=0.93) and the volume repaid after exercise in both groups, there were no differences between the two groups. Conclusions: Exercise-induced metabolic vasodilation is in part dependent on NO release. Hypercholesterolemia impairs NO-mediated vasodilation, but is not associated with a reduction in exercise-induced hyperemia. This may indicate that multiple compensatory mechanisms are operative in skeletal muscle metabolic vasodilation.

Keywords: Regional blood flow; Cholesterol; Nitric oxide; Endothelial function; Vasoconstriction/vasodilation

1. Introduction

The vascular endothelium has been shown in recent years to play a central role in the regulation of vasomotor tone, and in the prevention of atherosclerosis by inhibiting thrombosis, inflammation and smooth muscle cell proliferation [1]. Numerous risk factors for atherosclerosis have been shown to be associated with endothelial dysfunction, which is usually assessed by endothelium-dependent vaso-motion [2]. Impaired vasodilation in response to stimulated release of endothelium-derived nitric oxide (NO) has been demonstrated in patients with hypertension, [3] diabetes mellitus, [4] hyperhomocysteinemia, [5] a history of smoking, [6] a family history of coronary artery disease, [7] and is more marked in those with multiple risk factors [8,9]. Endothelial function also appears to decline with advancing age [10].
Table 1
Clinical characteristics

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Hypercholesterolemic subjects (n=20)</th>
<th>Controls (n=20)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>28.8±6.3</td>
<td>24.7±6.5</td>
</tr>
<tr>
<td>Gender (female/male)</td>
<td>12/8</td>
<td>11/9</td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>22.3±2.3</td>
<td>22.0±2.2</td>
</tr>
<tr>
<td>Waist:hip ratio</td>
<td>0.81±0.07</td>
<td>0.83±0.06</td>
</tr>
<tr>
<td>Forearm volume (ml)</td>
<td>862±211</td>
<td>904±205</td>
</tr>
<tr>
<td>Mean arterial blood pressure (mmHg)</td>
<td>83.1±11.8</td>
<td>79.0±7.0</td>
</tr>
<tr>
<td>Family history of IHD</td>
<td>4</td>
<td>0</td>
</tr>
<tr>
<td>No. using oral contraceptive</td>
<td>6</td>
<td>2</td>
</tr>
</tbody>
</table>

* Data is mean±SD. Abbreviation: IHD=ischemic heart disease.

Impaired endothelium-dependent NO-mediated vasodilation has also been documented in hypercholesterolemia in response to acetylcholine (ACh), [11–14] methacholine, [15,16] serotonin, [17] substance P [18] and shear stress, [19] suggesting that the abnormality is not linked to a single receptor-mediated pathway [2]. This dysfunction may occur at an early age and is detectable before the onset of overt vascular disease [19]. Improvement of endothelial function has been achieved with cholesterol-lowering, [17] l-arginine supplementation, [20] the addition of essential cofactors of endothelial NO synthase (eNOS), [21,22] and reduction of reactive oxygen species, [23,24] all of which improve NO bioavailability.

Hypercholesterolemia is an important risk factor for atherosclerosis, [25] and its treatment has been shown to improve survival and reduce morbidity in patients with coronary artery disease [26]. In these trials cholesterol-lowering was also shown to reduce the need for revascularization, and more recent studies have shown that this treatment decreased myocardial ischemia, [27] exercise-induced angina, [28] and improved exercise-induced coronary vasomotion [29].

Recent evidence suggests that NO is important in the regulation of resting skeletal muscle blood flow, [30] in the hyperemia after ischemia [31] and in exercise-induced metabolic vasodilation (functional hyperemic blood flow, FHBF) in humans [32–36]. Katz et al. also demonstrated that NO-mediated FHBF is impaired in patients with congestive cardiac failure [34]. Although receptor-mediated and shear stress induced NO-dependent vasodilation is impaired in hypercholesterolemia, it is unclear whether metabolic vasodilation in human skeletal muscle is reduced. Recent data in the hypercholesterolemic mouse model suggests that this may be so [37]. We therefore sought to determine if young, otherwise healthy subjects with hypercholesterolemia had impaired vasodilator responses to a metabolic stimulus, ACh, and sodium nitroprusside (SNP), and, if so, whether this was due to reduced NO bioavailability.

2. Methods

2.1. Subjects

Forty subjects (20 control, 20 with hypercholesterolemia) with a mean age of 26.7±6.7 years (mean±SD, 23 female, 17 male) were recruited by advertisement for this study. Subjects were defined as hypercholesterolemic if screening total cholesterol and low density lipoprotein-cholesterol (LDL) levels were above the 75th percentile for age and gender based on the National Heart Foundation of Australia Risk Factor Prevalence Study [38]. Their clinical characteristics are shown in Table 1 and their lipid profiles in Table 2. All subjects were screened for cardiovascular risk factors, cardiovascular disease or other major illness by medical history, physical examination and fasting lipid profile. Subjects were excluded if they had any other risk factor for ischemic heart disease (apart from family history), cardiovascular disease, major noncardiac disease or any abnormality on physical examination (including a discrepancy of ≥10 mmHg of blood pressure between the upper limbs). Subjects on cardiovascular medications were excluded from either group, though females on estrogen

Table 2
Lipid profile

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Hypercholesterolemic subjects (n=20)</th>
<th>Controls (n=20)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total cholesterol (mmol/l)</td>
<td>6.9±1.4&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.6±0.6</td>
</tr>
<tr>
<td>LDL (mmol/l)</td>
<td>4.9±1.6&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.7±0.6</td>
</tr>
<tr>
<td>HDL (mmol/l)</td>
<td>1.5±0.3</td>
<td>1.6±0.4</td>
</tr>
<tr>
<td>LDL-HDL ratio</td>
<td>3.7±2.0&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.9±0.6</td>
</tr>
<tr>
<td>Triglycerides (mmol/l)</td>
<td>1.3±0.7&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.8±0.2</td>
</tr>
<tr>
<td>Glucose (mmol/l)</td>
<td>4.8±0.4</td>
<td>4.9±0.4</td>
</tr>
<tr>
<td>Lp(a) (mg/l)</td>
<td>489±466</td>
<td>303±200</td>
</tr>
</tbody>
</table>

<sup>a</sup> Data is mean±SD. Comparison of hypercholesterolemic vs. control subjects.

<sup>b</sup> P<0.005.
<sup>c</sup> P<0.001.
<sup>d</sup> P<0.0001.
containing oral contraceptives were included. The study was approved by the National Health and Medical Research Council of Australia and the Human Research Ethics Committee of Monash Medical Centre, and all subjects gave their written informed consent. The investigation conforms to the principles outlined in the Declaration of Helsinki.

2.2. General methods

All subjects were examined in our vascular research laboratory in the morning after a light breakfast. The room was temperature controlled at 22–23°C, a quiet atmosphere prevailed and the lights were dimmed. Subjects were asked to refrain from caffeine containing food and drinks and alcohol for 12 h before the study. Aspirin and other nonsteroidal anti-inflammatory drugs were forbidden for a week prior to the study. After 10 min quiet, supine rest a 20-gauge, 5 cm polyethylene catheter (Cook, Brisbane, Australia) was introduced under local anesthesia into the brachial artery of the non-dominant upper limb. The procedure was carried out under aseptic conditions. The arterial cannula served as an infusion port for vasoactive agents and enabled blood pressure to be monitored directly and continuously. In order to establish a stable baseline all subjects were rested for at least 30 min after arterial line insertion before the first measurement was made. During this time isotonic glucose (5% Dextrose) was infused at a rate of 0.4 ml/min intraarterially (the same rate at which all drugs were subsequently infused).

2.3. Study design

To determine whether hypercholesterolemia impaired NO-dependent vasomotion we compared forearm blood flow (FBF) at rest, following isotonic exercise (FHBf), and in response to ACh, SNP and N\textsuperscript{\textomega}-monomethyl-L-arginine (L-NMMA) infusions between the two groups. Flow was measured using venous occlusion plethysmography. The metabolic stimulus consisted of rhythmic, isotonic exercise at the wrist. Maximal flexion–extension wrist movement at 45 cycles per min was encouraged and timed with a metronome. Reproducibility of FHBf in response to this stimulus has already been established in our laboratory [35].

The study design is indicated in Fig. 1. Resting and stimulated FBF and FHBf were assessed with vehicle infusion (isotonic glucose infusion), then repeated with L-NMMA infusion. After the initial baseline resting flow all subjects received ACh. The response to ACh was reassessed during L-NMMA infusion. SNP was also given before and after L-NMMA. Baseline flow was reestablished after each intervention.

2.4. Drug infusion protocols

Acetylcholine chloride (ACh, Miochol, Iolab Pharmaceuticals, Sydney, Australia), an agent which causes vasodilation by release of endothelial-derived NO [30] and endothelium-derived hyperpolarizing factor, [39] was infused via the brachial artery for 5 min in a dose of 30 μg/min as previously described [31,40]. SNP (Faulding, Melbourne, Australia), a NO donor which results in direct vascular smooth muscle relaxation, was administered via the brachial artery for 5 min at a rate of 1 μg/min as described in previous studies, [31,40] to assess the response to an endothelial-independent vasodilator. These drug doses have previously been shown in our laboratory not to have systemic effect [40]. Only one dose of each agonist was given in order to optimise the length of the protocol to allow for all the other measurements required. L-NMMA (Clinalfa, Switzerland) inhibits the production of NO by competing with L-arginine for the enzyme NOS [30]. It has been shown in several studies to reduce resting and stimulated FBF by up to 50% [30,32,34–36] and inhibit metabolic vasodilation by between 10 and 50\% [32–36]. L-NMMA was infused in a dose of 2 mg/min for at least 10 min, as in previous studies [32,35].

All drugs were diluted in an isotonic glucose solution (5% Dextrose) and were infused at a rate of 0.4 ml/min using a syringe pump (Terumo Corporation, Tokyo, Japan), the same rate as the vehicle infusion during the initial resting and exercise-induced blood flows.

2.5. Hemodynamic measurements

FBF was measured in the nondominant arm by venous

Study Protocol

<table>
<thead>
<tr>
<th>Basal</th>
<th>ACh</th>
<th>Basal</th>
<th>Exercise</th>
<th>Basal</th>
<th>SNP</th>
<th>Basal</th>
<th>ACh</th>
<th>Basal</th>
<th>Exercise</th>
<th>Basal</th>
<th>SNP</th>
</tr>
</thead>
</table>

Fig. 1. Study Design. Time line of the study measurements. Abbreviations: ACh = acetylcholine; SNP = sodium nitroprusside; L-NMMA = N\textsuperscript{\textomega}-monomethyl-L-arginine. Basal represents a resting measurement.
occlusion plethysmography using calibrated mercury-in-silastic strain gauges (Hokanson, Bellevue, WA, USA) and expressed in milliliters per 100 milliliters of forearm tissue per min, as previously described [40]. Forearm vascular resistance (expressed as units indicating mmHg/ml/100 ml of tissue/min) was calculated from mean arterial blood pressure and FBF, while minimum resistance after exercise was calculated from mean arterial blood pressure and peak FHBF.

To assess resting blood flow, measurements were taken for at least 2 min and an average of a minimum of five results were used for analysis. Exercise-induced FHBF was measured continuously for 5 min after the metabolic stimulus to examine the time course of recovery and volume of blood repaid during the hyperemic response, and this commenced immediately after the cessation of exercise. Short venous occlusion cycles were used for at least the first minute, with 12 s cycles thereafter. Peak FHBF was determined, and usually occurred in the first 10–15 s after completion of the workload. The blood volume (“debt”) repaid during the first minute and subsequent 4 min after exercise was calculated from the area-under-the-curve.

2.6. Lipid measurements

Total cholesterol, high density lipoprotein (HDL) and triglyceride concentrations were measured enzymatically. LDL was calculated according to the Friedewald formula. Lipoprotein (a) [Lp(a)] was measured by a modified Behring commercial latex enhanced immunoephelometric method. The upper limit reference range in our laboratory by 12% in the control group, by coinfusion with L-NMMA (clinical) variables. Multivariate analysis was performed to determine the relation between the dependent variables. Univariate analysis using simple linear regression was used to determine the relation between the dependent variables (FHBF, ACh and SNP FBF responses) and independent (clinical) variables. Multivariate analysis was performed using multiple linear regression to determine the best combination of predictor variables. Statistical significance was accepted where \( P < 0.05 \).

3. Results

The two groups were well matched for age, sex, and clinical and morphometric characteristics (Table 1). The hypercholesterolemic group had higher total and LDL-cholesterol levels, LDL:HDL ratio and triglyceride levels than the control group (Table 2).

3.1. Resting hemodynamics

Resting FBF before any intervention was the same in both groups (2.1±0.2 vs. 2.1±0.2, mean±SE, \( P = 0.79 \)). Resting forearm vascular resistance was also similar in the hypercholesterolemic and control groups (45.1±3.5 vs. 42.8±4.4 units, respectively, \( P = 0.68 \)). There was no difference in mean arterial blood pressure at baseline between the two groups (83.1±2.6 vs. 79.0±1.6 mmHg, respectively, \( P = 0.19 \)). Infusion of L-NMMA resulted in a similar 33±4% reduction of resting FBF in hypercholesterolemic subjects and 40±4% reduction in controls (\( P = 0.17 \), Fig. 2). There was a corresponding 60±8% vs. 82±11% increase in resting forearm vascular resistance, respectively, with L-NMMA (\( P = 0.12 \), Fig. 2). Mean arterial blood pressure was not affected during these measurements by the L-NMMA infusion.

3.2. Stimulated endothelium-dependent and independent responses

ACh infusion increased resting FBF in both groups, but the increment was less in hypercholesterolemic subjects compared to controls (\( P = 0.03 \), Fig. 3). The percentage change in FBF in response to ACh was also less in hypercholesterolemic subjects (452±92% vs. 741±104%, \( P = 0.04 \)). The FBF response to ACh was attenuated similarly, by 10% in the hypercholesterolemic group and by 12% in the control group, by confusion with L-NMMA (Fig. 3), though this reduction did not achieve significance in either group.

SNP infusion increased resting FBF in both groups, but the increment was less in the hypercholesterolemic subjects compared to controls (\( P < 0.01 \), Fig. 3). The percentage change in resting FBF with SNP was slightly, though not significantly, lower in the hypercholesterolemic subjects (155±19% vs. 197±18%, \( P = 0.11 \)). The FBF response to SNP before and after L-NMMA infusion was assessed in 19 hypercholesterolemic subjects and in 13 controls, and was not affected by L-NMMA (Fig. 3). Mean arterial blood pressure was not affected by either ACh or SNP infusions.

3.3. Metabolic vasodilation

Peak FHBF in response to exercise was similar in the hypercholesterolemic and control groups (13.1±1.0 vs. 13.2±1.0 ml/100 ml/min, respectively, \( P = 1.0 \), Fig. 4). The blood volume repaid during the first minute after exercise was also similar in the hypercholesterolemic and control groups (\( P = 0.46 \), Fig. 5). The blood volume repaid during the 5 min after exercise was also similar in both groups (\( P = 0.32 \), Fig. 5). Mean arterial blood pressure and
Fig. 2. Resting forearm hemodynamics, and the response to L-NMMA. L-NMMA infusion decreased FBF and increased forearm vascular resistance to a similar extent in both groups. Clear bars: resting hemodynamics in hypercholesterolemics. Speckled bars: effect of L-NMMA in hypercholesterolemics. Hatched bars: resting hemodynamics in controls. Cross hatched bars: effect of L-NMMA in controls. * $P<0.0001$ pre vs. post L-NMMA.

Fig. 3. FBF response to ACh and SNP infusions, and the response to L-NMMA. Left panel: the response to ACh in ml/100 ml/min: L-NMMA infusion decreased the response to a similar extent in both groups, though the reduction was not significant. Right panel: the response to SNP in ml/100 ml/min. L-NMMA did not significantly reduce the response in either group. Clear bars: ACh and SNP response in hypercholesterolemics. Speckled bars: effect of L-NMMA in hypercholesterolemics. Hatched bars: ACh and SNP response in controls. Cross hatched bars: effect of L-NMMA in controls. *$P<0.05$ and **$P<0.01$ for the comparison between the groups.
to 91.4 ± 2.0 mmHg (P < 0.0001), while in control subjects blood pressure increased from 80.6 ± 1.7 to 85.6 ± 1.8 mmHg (P < 0.001).

3.4. Predictors of FBF responses

There was no relationship between resting FBF, peak hyperemic FHB, or the volume of blood repaid after 1 and 5 min and any of the lipid or clinical parameters. The lipid and clinical variables did not predict the percentage change in FBF with L-NMMA either.

Univariate analysis revealed that the best predictors of the ACh FBF response were female gender (r = 0.38, P = 0.02) and HDL-cholesterol (r = 0.34, P = 0.03). Multiple regression analysis revealed that the best combination of predictors of the ACh response was LDL, HDL and gender (R² = 0.24, P < 0.02). Though weakly inversely correlated, total cholesterol (r = −0.30, P = 0.06) and LDL-cholesterol (r = −0.30, P = 0.07), were the best univariate predictors of
both these parameters after inhibition of NO with L-NMMA. Moreover, there was no apparent relationship in our study between lipid parameters and FBF at rest or in response to exercise.

Our findings are consistent with those of Creager et al. who observed no reduction in reactive hyperemia after ischemia in hypercholesterolemia, though the responses to methacholine and SNP were diminished [15]. Sorensen et al. also noted no difference in reactive hyperemia after ischemia in hypercholesterolemia, but detected impaired NO-dependent, flow-mediated dilation [19]. The apparent discrepancy between the preservation of resistance vessel function (reactive and functional hyperemia) and the observed conduit vessel dysfunction (flow-mediated dilation) may relate to the dependence of flow-mediated dilation on NO release [41]. However, inhibition of NO production with L-NMMA was not examined in either of these studies. In contrast, control of reactive and functional hyperemia appears to be more complex [42]. Several factors may contribute to these responses including vasodilator prostanoids, metabolites, ATP-sensitive K⁺ channels and myogenic factors [35,42,43].

The findings of the present study extend those of previous related studies [15,19] by demonstrating that functional hyperemia is preserved in the presence of impaired responses to NO-mediated vasodilators, despite evidence that NO contributes to functional hyperemia in these subjects. While similar factors, such as accumulation of vasodilator metabolites, may contribute to both reactive hyperemia after ischemia and functional hyperemia associated with exercise, there are significant differences between these responses. For example, myogenic responses are thought to be more important to reactive compared to functional hyperemia, and the relative importance of endothelial factors to these responses may be different [35,44,45]. In addition, functional hyperemia is more physiologically relevant than the response to either ischemia or pharmacological stimuli.

Whether hypercholesterolemia affected other factors involved in metabolic vasodilation has not been addressed by this study. This important physiological function is regulated by a number of overlapping mediators, and inhibition of one may result in compensatory upregulation of another [42,43]. It has recently been demonstrated in an experimental model that while ATP-sensitive K⁺ channels are critical in coronary vasodilation during exercise, flow still increased substantially with exercise [43]. Blockade of ATP-sensitive K⁺ channels, followed by blockade of adenosine receptors, and finally inhibition of NO production resulted in incremental reductions of metabolic vasodilation. With all three mechanisms inhibited both flow at rest and during exercise was below control resting level [43]. Therefore, multiple vasodilator pathways may need to be inhibited in young, otherwise healthy hypercholesterolemic subjects to show a reduction in metabolic vasodilation. Alternatively, metabolic vasodilation
may only be impaired if multiple risk factors for atherosclerosis are present [8,9].

4.2. Pharmacologic responses

Impaired ACh-induced vasodilation associated with hypercholesterolemia in our study is consistent with previous investigations utilising receptor-mediated, NO-dependent vasodilation as a marker of endothelial function. We also demonstrated an impaired vasodilator response to SNP in hypercholesterolemic subjects. Others have also shown that hypercholesterolemia is associated with reduced response to nitrovasodilators, [14,15,19] though this has not been a consistent finding [11,12,16]. Interestingly, a recent study in which transient hypertriglyceridemia was induced in healthy males there was impaired nitroglycerin-induced and flow-mediated vasodilation [46]. The abnormal responses to agonists that stimulate NO production and release as well as to NO donors suggests that the defect in hypercholesterolemia lies beyond eNOS.

Although L-NMMA attenuated the ACh response in both groups by approximately 10%, this did not achieve significance. These findings in hypercholesterolemia are similar to those of Casino et al., however they also found a significant reduction in the ACh response with L-NMMA in their control group [12]. Importantly, the reduction in the NO component of resting FBF and FHBF was consistent in our study, and of similar magnitude to that found in previous studies in which the response to ACh was also attenuated by L-NMMA [30,32,33]. Moreover, the dose of L-NMMA used in our study would be expected to inhibit NO production [30,32]. It is possible that the impaired response to ACh may be due to factors other than NO, as it has recently been demonstrated that endothelium-derived hyperpolarizing factor-mediated relaxation may be impaired, and vasoconstrictor prostanoids may be augmented in hypercholesterolemia in humans [47,48]. Another possibility is that the design of our study may have limited our ability to detect a reduction in the ACh response after L-NMMA. We used only one dose of ACh on each occasion as our primary objective was assessment of metabolic vasodilation, but recent evidence suggests that several doses of ACh are required after NO inhibition to attenuate the response [49]. The inability of L-NMMA to inhibit ACh-mediated vasodilation has been a consistent finding in our laboratory in response to a single dose of ACh [35]. This suggests that the first dose of ACh may only release preformed stores of NO-containing factors from the endothelium [49]. It is possible, therefore, that a dose–response curve to ACh before and after L-NMMA may have detected significant attenuation of the response.

4.3. Mechanisms of impaired NO responsiveness

A number of potential mechanisms have been proposed to explain the observed impairment in NO bioavailability associated with atherosclerosis and with risk factors for atherosclerosis, such as hypercholesterolemia [2]. These include attenuation of receptor-mediated eNOS activation, relative reduction of L-arginine as a substrate for eNOS, decreased eNOS expression, diminished tetrahydrobiopterin availability, and increased destruction of NO by reactive oxygen species [2]. Although alteration of muscarinic receptor-mediated eNOS stimulation would explain the reduced ACh FBF response, this does not explain the impaired response to the NO donor SNP. As ACh and SNP both induce vasodilation by NO-mediated vascular smooth muscle relaxation, increased breakdown of NO would explain our results. It is also possible that a defect in smooth muscle response per se may be responsible for the observed findings. However, it may be that hypercholesterolemia may affect more than one of these mechanisms simultaneously.

4.4. Study limitations

Although we have demonstrated that NO-dependent metabolic vasodilation is not impaired in young volunteers with hypercholesterolemia, it is possible that older subjects with age related decline in endothelial function, [10] or those with multiple risk factors for atherosclerosis may demonstrate impaired NO-dependent metabolic vasodilation [9].

Another potential limitation of this study is the inclusion of a small number of women taking estrogen containing oral contraceptives. Fluctuations in circulating endogenous estrogen levels has been shown to effect endothelial function [50]. For ethical reasons we did not request cessation of that medication for the study. Nevertheless, the number of women taking oral contraceptives in each group was similar, and their use did not predict ACh or SNP FBF responses on univariate analysis.

4.5. Clinical implications

The effect of endothelial dysfunction secondary to hypercholesterolemia on physiological functions, such as exercise hyperemia, has largely been unexplored, but is of critical importance to the understanding of ischemic syndromes. The young hypercholesterolemic subjects in our study, carefully screened to exclude other risk factors and overt atherosclerosis, did not demonstrate reduced metabolic vasodilation despite impaired ACh and SNP responses. However, studies in the coronary circulation of older patients with multiple risk factors for atherosclerosis have shown impairment of metabolic vasodilation [9]. Further studies will be required to determine whether improvement in endothelial function with cholesterol-lowering will improve metabolic vasodilation and reduce ischemia in advanced disease.
5. Conclusion

This study has shown that young, otherwise healthy hypercholesterolemic subjects have preserved resting skeletal muscle blood flow and vasodilation to a metabolic stimulus despite impaired ACh and SNP responses. It is therefore likely that the various factors involved in metabolic vasodilation are not affected by hypercholesterolemia early in the disease process, or that there is compensatory upregulation of some of these mechanisms in the early stages of atherosclerosis.

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References


