Impaired flow-mediated vasoactivity during post-prandial phase in young healthy men

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Impaired flow-mediated vasoactivity during post-prandial phase in young healthy men

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Abstract

Impaired flow-mediated vasodilation in large arteries is an expression of endothelial dysfunction and an established marker of early atherosclerosis. Post-prandial lipemia can induce an impairment of the endothelial function. The aim of our study was to evaluate the effects of post-prandial phase on flow-mediated vasodilation in a group of ten young (23 ± 2 years) healthy men without cardiovascular risk factors, who underwent an oral fat-loading test. Flow-mediated vasodilation of the brachial artery and serum lipid profile were assessed under fasting conditions and 2, 4, 6 and 8 h after a high-fat meal. Triglycerides increased from 0.6 ± 0.2 fasting to 1.1 ± 0.5 and 1.3 ± 0.6 mmol/l at the 2nd and 4th hour (both P < 0.01), and decreased thereafter. Flow-mediated vasodilation fell significantly from 14.5 ± 6.6% fasting to 3.5 ± 1.5% and 4.0 ± 2.2% at the 2nd and 4th hour (both P < 0.01), and returned to the basal values at the 6th and 8th hour. A strong inverse correlation was observed between the area under the incremental curve of post-prandial triglycerides (i.e. after subtraction of baseline triglycerides) and the area under the decremental curve of post-prandial flow-mediated vasodilation (r = -0.70, P = 0.025). No association was found between post-prandial vasodilation changes and fasting triglycerides, other lipid parameters or insulin. We conclude that a transient post-prandial impairment in brachial artery flow-mediated vasodilation is evident in young healthy men after a high-fat meal, and is closely associated with triglyceride levels. These data provide support for a role of post-prandial phase in vascular regulation in young healthy subjects. © 2000 Elsevier Science Ireland Ltd. All rights reserved.

Keywords: Vasodilation; Triglyceridemia; Arteries; Brachial; Post-prandial lipemia; Endothelium

1. Introduction

Endothelial dysfunction represents one of the earliest events in the development of atherosclerosis, and is thought to reflect a functional impairment of the endothelium before morphological changes can be detected [1]. Endothelial dysfunction has been described in association with several cardiovascular risk factors, including diabetes [2], hypertension [3], smoking [4,5], hypercholesterolemia [6], and hyperhomocysteinemia [7]. In response to various stimuli, including shear stress from flowing blood, the endothelium releases nitric oxide, which causes smooth muscle contraction and arterial dilation [8]. Therefore, flow-mediated vasodilation of the brachial artery is considered a measure of endothelial function [8,9]. Noninvasive techniques for the assessment of endothelial function, such as flow-mediated vasodilation, are promising alternatives to the traditional invasive and expensive methods.

Several years ago, Zilversmit et al. suggested the hypothesis of considering post-prandial phase as a risk condition for atherosclerosis [10]. More recently, some studies focused on the impact of triglyceride levels on endothelial function. Lundman et al. observed that a non-physiological infusion of a triglyceride emulsion induced a loss of vascular reactivity mediated by both endothelium-dependent and endothelium-independent mechanisms [11]. Vogel et al. demonstrated that endothelial function is impaired in a sample of middle-aged men and women after a high-fat meal; in that
study, transient endothelial dysfunction showed a significant correlation with post-prandial serum triglycerides [12].

The aim of the present study was to evaluate whether serum lipid changes induced by a high-fat meal are able to modify flow-mediated vasodilation even in very young, healthy men without cardiovascular risk factors, and to evaluate the correlation between arterial vasoactivity and lipid modifications.

2. Patients and methods

2.1. Study protocol

Ten physically active healthy young men (mean age 23 ± 2 years) without cardiovascular risk factors (diabetes, hypertension, overweight, hyperlipemia, smoking and family history for early-onset cardiovascular disease) were recruited among the staff of our hospital. Four weeks before the study, patients met with a dietitian and were instructed to consume a weight-maintaining typical Mediterranean diet with fat < 10% of total calories, saturated fats < 7% and a monounsaturated/saturated fat ratio > 1. Dietary habits were checked by questionnaire.

Data collection began at 08:00 h after 14-h fasting. Before administration of the fatty meal, blood was drawn for the determination of total cholesterol, low density lipoprotein (LDL) cholesterol, high density lipoprotein (HDL) cholesterol, HDL2 cholesterol, HDL3 cholesterol, triglycerides, LDL size, lipoprotein (a) and insulin. Flow-mediated vasodilation of the brachial artery was assessed at the same time. The standardized high-fat meal consisted of whipping cream, liquid chocolate and non-fat dry milk and contained 65 g of fat, 25 g of carbohydrates, 6 g of proteins per m2 body surface area [13]. The corresponding caloric intake was 700 kcal/m2. Thereafter, blood samples were obtained every 2 h for 8 h and analyzed for serum lipids and insulin. Ultrasonographic assessment of brachial artery flow-mediated vasodilation was repeated at the same intervals. All subjects gave their written informed consent to the protocol, which was approved by the Ethics Committee of our institution.

2.2. Ultrasonographic assessment of flow-mediated vasodilation

Flow-mediated vasodilation was assessed by 2-dimensional ultrasonography of the brachial artery according to the method described by Plotnick et al. [14]. The measurements were performed in supine position on the left arm, after 10–20 min resting in a quiet, dark room with a temperature of 22°C. The brachial artery was scanned longitudinally just above the antecubital crease using a 10 MHz probe (ESAOTE Challenge Sim 7000, Florence, Italy). Diameter of the brachial artery was measured at the R wave of the electrocardiogram, on the interface between media and adventitia of the anterior and posterior wall. Gain settings were optimized to identify the lumen and the vessel wall interfaces and were not modified during the examination. Hyperemia was induced by inflation of a pneumatic cuff (12.5 cm wide) at 230–250 mmHg for 4 min on the most proximal portion of the upper arm. Arterial diameter measurement was repeated 45–60 s after sudden deflation of the cuff. Tracings were recorded on videotape and read by one investigator, who was unaware of the subject’s clinical data and temporal sequence. The average of three measurements of basal and post-hyperemia diameter was used for the analysis. Flow-mediated vasodilation was expressed as the relative increase in brachial artery diameter during hyperemia, and defined as 100 × [(post-hyperemia diameter−basal diameter)/basal diameter]. Blood flow velocity was measured by Doppler technique at baseline and immediately after cuff release. Blood flow was determined as arterial cross sectional area (π × r2) times mean Doppler velocity. The intra-observer between-occasion reproducibility of flow-mediated vasodilation in our laboratory was assessed in 21 subjects examined 2 days apart. The mean ± SD difference between the two examinations was 1.0 ± 1.5%.

2.3. Lipid parameters

Total cholesterol, triglycerides and HDL cholesterol were determined by enzymatic colorimetric method (Dimension Autoanalyzer, DADE Inc., Newark, NJ), and LDL cholesterol was calculated from the Friedewald equation [15]. Plasma lipoprotein (a) concentration was measured by enzyme-linked immunosorbent assay. HDL2 cholesterol and HDL3 cholesterol concentrations were determined after precipitation with two different reagents containing polyethylene glycol (Immuno AG, Vienna, Austria). An aliquot of plasma (blood anticoagulated with EDTA) was used to determine LDL size by gradient gel electrophoresis according to Rainwater et al. [16]. A 2–16% polyacrilamide gel was prepared; samples for seeding were pre-incubated, stained with Sudan Black, and seeded with thyreoglobulin, ferritin, catalase, lacticodehydrogenase, albumin and latex of a known size to be used as standard migration distances. The migration distances were read using densitometry (590 nm). A quadratic equation (polynomial regression of Stokes) was used to convert migration distance into particle diameter. The estimated diameter for the major peak in each scan was identified as the LDL peak particle diameter. Insulin was determined in duplicate by radioimmunoassay.
2.4. Statistical analysis

The Kolmogorov–Smirnov algorithm was used to determine whether each variable had a normal distribution. Parameters are expressed as mean ± SD, except for lipoprotein (a) which is expressed as median (25th–75th percentile). Analysis of variance for repeated measurements was used to assess differences among different hours. For all parameters which showed significant changes during the 8 h of measurement as compared with fasting levels, the areas under the incremental or decremental post-prandial curves (ΔAUC, i.e. the increment or decrement after subtraction of baseline values) were calculated by the trapezoidal method. Pearson’s correlation coefficients were used to assess the relation of ΔAUC of brachial artery flow-mediated vasodilation with (a) ΔAUC of serum triglycerides, (b) ΔAUC of peak arterial flow during reactive hyperemia and (c) ΔAUC of serum insulin.

3. Results

All study subjects had normal body weight (body mass index 23 ± 2 kg/m², waist-hip ratio 0.79 ± 0.1) and blood pressure values (114/72 ± 8/5 mmHg). Table 1 shows serum lipids and ultrasonographic parameters at baseline and 2, 4, 6 and 8 h after the meal. As compared to pre-prandial values, serum triglycerides showed a significant increase 2 and 4 h after the meal, and returned to baseline levels thereafter. No significant change was observed in the other lipid parameters. Flow-mediated vasodilation showed a marked reduction at the 2nd and 4th hour, and returned to the basal values at the 6th and 8th hour. Post-prandial changes in flow-mediated vasodilation and serum triglycerides are illustrated in Fig. 1. No significant changes in post-hyperemic brachial artery flow, either expressed in absolute values or after subtraction of pre-prandial values, were observed during the 8 h of measurement. Serum insulin increased 2 h after the high-fat meal, and returned to baseline values thereafter.

As shown in Fig. 2, ΔAUC of post-prandial percent flow-mediated vasodilation of the brachial artery showed a close inverse correlation with ΔAUC of post-prandial serum triglycerides ($r = -0.70$, $P = 0.025$). Changes in flow-mediated vasodilation showed no significant correlation with the changes in serum insulin ($r = -0.07$, $P = n.s.$), and with the other lipid parameters and their post-prandial changes (all $P = n.s.$). Similarly, no significant association was found between flow-mediated vasodilation changes and fasting triglyceride levels ($r = -0.34$, $P = 0.32$), and between fasting vasoactivity and fasting lipid levels (all $P = n.s.$).

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Table 1
Serum lipids and vasoactivity parameters at baseline and after high-fat meal

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Baseline</th>
<th>2 h</th>
<th>4 h</th>
<th>6 h</th>
<th>8 h</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total cholesterol (mmol/l)</td>
<td>3.70 ± 0.33</td>
<td>3.54 ± 0.54</td>
<td>3.57 ± 0.54</td>
<td>3.54 ± 0.51</td>
<td>3.65 ± 0.54</td>
</tr>
<tr>
<td>LDL* cholesterol (mmol/l)</td>
<td>2.00 ± 0.51</td>
<td>1.81 ± 0.59</td>
<td>1.76 ± 0.67</td>
<td>1.76 ± 0.54</td>
<td>1.91 ± 0.38</td>
</tr>
<tr>
<td>HDLb cholesterol (mmol/l)</td>
<td>1.16 ± 0.18</td>
<td>1.16 ± 0.15</td>
<td>1.24 ± 0.15</td>
<td>1.19 ± 0.15</td>
<td>1.29 ± 0.10</td>
</tr>
<tr>
<td>HDLc cholesterol (mmol/l)</td>
<td>0.31 ± 0.15</td>
<td>0.25 ± 0.12</td>
<td>0.31 ± 0.12</td>
<td>0.31 ± 0.15</td>
<td>0.31 ± 0.15</td>
</tr>
<tr>
<td>LDLc cholesterol (mmol/l)</td>
<td>0.82 ± 0.12</td>
<td>0.82 ± 0.12</td>
<td>0.85 ± 0.15</td>
<td>0.82 ± 0.10</td>
<td>0.95 ± 0.18</td>
</tr>
<tr>
<td>Triglycerides (mmol/l)</td>
<td>0.63 ± 0.21</td>
<td>1.11 ± 0.48*</td>
<td>1.35 ± 0.58*</td>
<td>1.00 ± 0.84</td>
<td>0.66 ± 0.37</td>
</tr>
<tr>
<td>LDL size (mm)</td>
<td>28.2 ± 2.1</td>
<td>28.3 ± 2.1</td>
<td>28.0 ± 2.2</td>
<td>28.3 ± 2.3</td>
<td>29.0 ± 2.1</td>
</tr>
<tr>
<td>Lipoprotein (a) (mg/dl)</td>
<td>6.3 (3.4–15.7)</td>
<td>5.8 (2.8–15.5)</td>
<td>5.4 (2.6–14.7)</td>
<td>5.9 (3.0–15.9)</td>
<td>6.2 (3.3–16.3)</td>
</tr>
<tr>
<td>Insulin (mU/l)</td>
<td>6.5 ± 2</td>
<td>17.2 ± 5*</td>
<td>7.7 ± 2</td>
<td>6.0 ± 2</td>
<td>5.1 ± 2</td>
</tr>
<tr>
<td>Brachial artery diameter (mm)</td>
<td>3.91 ± 0.6</td>
<td>4.09 ± 0.6</td>
<td>3.99 ± 0.5</td>
<td>4.03 ± 0.7</td>
<td>3.84 ± 0.4</td>
</tr>
<tr>
<td>Flow-mediated vasodilation (%)</td>
<td>14.5 ± 6.6</td>
<td>3.5 ± 1.5*</td>
<td>4.0 ± 2.2*</td>
<td>12.5 ± 5.9</td>
<td>15.9 ± 6.5</td>
</tr>
<tr>
<td>Brachial artery flow at baseline (ml/min)</td>
<td>102 ± 30</td>
<td>103 ± 37</td>
<td>102 ± 39</td>
<td>111 ± 46</td>
<td>94 ± 31</td>
</tr>
<tr>
<td>Post-hyperemic brachial artery flow (ml/min)</td>
<td>366 ± 88</td>
<td>364 ± 112</td>
<td>372 ± 78</td>
<td>383 ± 127</td>
<td>362 ± 72</td>
</tr>
</tbody>
</table>

* LDL, low density lipoprotein.

b HDL, high density lipoprotein.

c Data are expressed as mean ± SD and median (25th–75th percentile).

* $P < 0.05$ versus baseline.
men with normal fasting serum triglycerides, and no (a) during the post-prandial phase in young healthy cholesterol, HDL3 cholesterol, LDL size or lipoprotein under the incremental curve of serum triglycerides. during the 8 h following a high-fat meal and the simultaneous area curve of percent flow-mediated vasodilation of the brachial artery Fig. 2. Inverse correlation between the area under the decremental present study, we found no significant changes in HDL2 mechanisms operating in the post-prandial phase. In the rule out the possible influence of several other mecha- and flow-mediated vasodilation impairment does not served in the present study between triglyceride increase motriglyceridemic subjects. The strong relation ob- association was found in our healthy sample of nor- dependent vasodilation is controversial [23,24], and no the relation of fasting triglycerides with endothelium-esters. At variance with post-prandial triglyceridemia, [20] and penetrate the vessel wall within the suben- density lipoproteins, are taken up by the macrophages [13,17], and with progression of carotid artery intima-media thickness [18,19]. The post-prandial phase is deemed to be atherogenic because triglyceride-rich lipoproteins, including chylomicron remnants and very low density lipoproteins, are taken up by the macrophages [20] and penetrate the vessel wall within the subendothelial space [21,22] after enrichment in cholesterol esters. At variance with post-prandial triglyceridemia, the relation of fasting triglycerides with endothelium-dependent vasodilation is controversial [23,24], and no association was found in our healthy sample of normotriglyceridemic subjects. The strong relation observed in the present study between triglyceride increase and flow-mediated vasodilation impairment does not rule out the possible influence of several other mechanisms operating in the post-prandial phase. In the present study, we found no significant changes in HDL2 cholesterol, HDL3 cholesterol, LDL size or lipoprotein (a) during the post-prandial phase in young healthy men with normal fasting serum triglycerides, and no relation was observed between changes in flow-mediated vasodilation and the other lipid parameters. However, we did not determine serum chylomicrons, chylomicron remnants, lipid hydroperoxides or other measures of oxidative lipid modification. In our study, the persistence of high triglyceride levels at the 6th hour despite the return toward normal values of flow-mediated vasodilation may lend support to this hypothesis. In this regard, Doi et al. observed that remnant lipoproteins contain a substantial amount of phospholipid hydroperoxides, capable of impairing flow-mediated vasodilation [25]. Moreover, antioxidant treatment with vitamin E in patients with increased levels of remnant lipoproteins reduces lipid peroxidation and increases arterial vasoactivity [26].

Abnormalities of endothelium-dependent arterial reactivity are widely recognized as an early marker of atherosclerosis. Impaired flow-mediated vasodilation has been described in diabetes mellitus [2], hypertension [3], hypercholesterolemia [6], hyperhomocysteinemia [7] and in association with active and passive smoking [4,5]. Furthermore, the clinical relevance of endothelium-dependent brachial artery vasodilation is documented by its close relation to acetylcholine-dependent coronary artery vasoactivity [27], thus indicating the systemic nature of endothelial dysfunction in atherosclerosis.

Reactive hyperemia is a multifactorial phenomenon, involving both vasodilator metabolites and myogenic influences [28]. We did not assess vasodilation in response to exogenous nitrates; thus, we cannot exclude definitely the possibility that the high-fat-induced impairment of vasodilation is by a non-endothelium-de- pendent mechanism. However, it has been shown that brachial artery response to hyperemia is dependent on nitric oxide bioavailability [4,8,9]. In our study, peak reactive flow in the post-prandial phase did not change as compared to fasting values, thus excluding the possi- ble influence on vasodilation of an altered flow pattern. Of note, Kugiyama et al. recently showed that triglyce- ride-rich remnant lipoproteins are independently associ- ated with endothelial dysfunction in epicardial coronary arteries in patients without angiographically proven vascular disease [29].

In the present study, arterial occlusion was induced above the examined brachial artery segment, while occlusion was below the site of investigation in the experi- ment which demonstrated the endothelium-dependent nature of flow-mediated arterial vasodilation [8]. De- spite the conceptual differences between the two ap- proaches, a subsequent study showed that also post-ischemic forearm vasodilation following proximal occlusion depends on endothelium-derived nitric oxide [9]. Moreover, in a direct comparison between the two methods, vasodilation induced by occlusion above the study site showed a significant direct relation with dilation induced by distal occlusion [30].
Some limitations of the present study deserve comment. First, we studied a relatively small numbers of subjects. Further investigations in larger numbers of young subjects are needed to confirm the negative impact of post-prandial lipemia on flow-mediated vasodilation in young healthy subjects. Secondly, the effect of a low-fat meal on flow-dependent vasodilation was not assessed in our study. However, previous investigators have consistently shown that low-fat meals do not induce changes in flow-mediated vasoactivity [12,14]. Moreover, we found that post-prandial insulin levels had no significant association with arterial vasodilation changes. Thirdly, we studied flow-mediated dilation in the brachial artery, while the clinical effects of impaired endothelial function are more prominent in the coronary arteries. Despite the close relation between endothelial function in the coronary and peripheral circulations [27], endothelial dysfunction may affect different arteries to a different degree.

Our findings support the hypothesis that, even in young healthy subjects free of cardiovascular risk factors, high-fat diets may lead to atherosclerosis through mechanisms at least in part independent of changes in cholesterol levels, by impairing endothelium-dependent vasoactivity. This impairment could be a marker of susceptibility to atherosclerosis in this group of subjects. Two recent studies reported decreased vascular reactivity following transient triglyceridemia [10,11]. The present study differs from the study by Vogel et al. [11] for two main reasons. (1) Our findings were obtained in a younger population (mean age 23 ± 2 years, vs. 39 ± 10 years in the study by Vogel et al.) thereby documenting the potentially atherogenic effect of a high-fat meal at a very young age. (2) We tested and excluded the effect on flow-mediated vasodilation of several other factors of the post-prandial phase, including lipoprotein (a), LDL size and HDL cholesterol fractions. At variance with Lundman et al. [10], who used the infusion of an artificial lipid emulsion, we employed a high-fat meal, thus testing the atherogenic effect of a saturated fat load (65 g/m²) which is not uncommonly served, especially at ‘fast-food’ restaurants. Further studies are needed to assess the mechanisms underlying the present findings, and to evaluate the influence of different factors of the post-prandial phase on endothelial function.

References


