Effect of statin versus fibrate on postprandial endothelial dysfunction: role of remnant-like particles

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Received 8 November 2000; accepted 25 January 2001

Abstract

Background: Postprandial lipemia is associated with endothelial dysfunction. Remnant-like particles (RLP) have been suggested to contribute to these adverse vascular effects. We investigated the effect of cerivastatin and gemfibrozil upon oral fat load induced changes in endothelial function and postprandial lipid profile in vivo. Methods: In a randomized cross-over trial, 15 healthy volunteers received cerivastatin (0.4 mg once daily), gemfibrozil (900 mg once daily) or placebo for 3 weeks. Lipid profiles and flow mediated dilation (FMD) were assessed before and 4 h after an oral fat load. Endothelium-independent dilation was tested after nitroglycerine 0.4 mg sublingual spray. Results: After the placebo period, the oral fat load induced an increase in triglycerides (TG) and RLP-cholesterol (RLP-C) (0.9 ± 0.0.7 and 0.08 ± 0.0.04 mmol/l, respectively) and a significant decrease in FMD (9.1 ± 3.4 to 4.3 ± 3.3%, P < 0.05). After gemfibrozil, TG increase was attenuated (0.5 ± 0.0.5 mmol/l), whereas RLP-C increase (0.05 ± 0.0.09 mmol/l) and FMD decrease (9.0 ± 3.8 to 5.2 ± 2.6%, P < 0.05) were not different from placebo therapy. Cerivastatin did not affect TG increase (0.7 ± 0.0.8 mmol/l). RLP-C increase (0.02 ± 0.0.07 mmol/l) and FMD (7.9 ± 2.6 to 8.4 ± 2.8%) change were attenuated significantly compared to placebo. Endothelium-independent vasodilatation remained unaltered throughout the protocol. Conclusion: Cerivastatin, but not gemfibrozil significantly reduces RLP-C increase after an oral fat load in combination with a reversal of fat-load induced endothelial dysfunction. The present data imply that lowering of RLP-C, rather than lowering of total TG levels, may contributes to the prevention of endothelial dysfunction after an oral fat load during statin use. © 2001 Elsevier Science B.V. All rights reserved.

Keywords: Endothelial function; Statins; Vasoconstriction/dilation

1. Introduction

Increasing experimental and clinical evidence suggests that triglyceride-rich lipoproteins (TRL) and in particular remnant-like particles (RLP), that circulate postprandially, may contribute to the atherosclerotic process, and subsequently to cardiovascular disease progression [1–4]. In line, coronary endothelial dysfunction, an emerging surrogate endpoint for cardiovascular morbidity [5], is negatively correlated to RLP-cholesterol (RLP-C) levels [6,7]. Recently, we and others have shown that an acute lipid load is associated with a consistent onset of endothelial dysfunction [8–10], reaching a maximal impairment 4 h after a standard fat load [10]. The latter time point coincides with the triglyceride (TG) and RLP-C peak upon fat loading [11,12]. In the present study we hypothesized that in particular RLP particles are responsible for the adverse vascular effects in the postprandial phase.

The currently accepted lipid-lowering strategies clearly differ in their effect on postprandial lipid metabolism. We previously demonstrated a significant decrease in RLP-C levels with only minor effects on TG levels during statin therapy in patients with heterozygous familial hypercholesterolemia [11]. In contrast, fibrates have been reported to significantly reduce TG levels, whereas their effect on RLP-C levels is unknown. In view of these divergent
effects on postprandial lipid profile, we assessed the effects of these lipid lowering drugs on both endothelial function and postprandial lipid metabolism. Using a double-blind, placebo-controlled cross-over design, flow-mediated dilatation before and after an oral fat load was evaluated in 15 healthy volunteers using either cerivastatin 0.4 mg once daily (o.d.), gemfibrozil 900 mg once daily (o.d.) or placebo.

2. Methods

2.1. Subjects

Fifteen healthy male volunteers, aged 18–36, participated in this study. All individuals, except one, who had an occasional borderline blood pressure elevation, were normotensive (systolic blood pressure < 140 mmHg and diastolic blood pressure < 90 mmHg), non-smoking and had no history of cardiovascular disease, or family history of premature vascular disease. All subjects had fasting TG and cholesterol concentrations below 2.0 and 6.0, mmol/l, respectively. The subjects refrained from using medication. The study was approved by the Medical Ethics Committee of the University Medical Center Utrecht and written informed consent was obtained from all participants. The investigation conforms with the principles outlined in the Declaration of Helsinki (Cardiovascular Research 1997;35:2–3).

2.2. Study design

A randomized, cross-over trial was performed comparing cerivastatin (0.4 mg o.d.), gemfibrozil (900 mg o.d.) and placebo. All subjects were assigned randomly to receive cerivastatin, gemfibrozil or placebo. After 3 weeks of treatment, each subject returned for evaluation of vascular function, followed by 4 weeks of washout. Three weeks after cross-over, the subjects were restudied, and the same measurements were performed.

The vasomotion study consisted of assessment of flow mediated dilation (FMD) and nitroglycerin-induced vasodilation (NTG; see below) before and after a standard oral fat load. The fat load consisted of 50 g fat/m² body surface in the form of whipped cream (40% fat) [13]. All subjects refrained from drinking caffeine-containing beverages and eating 12 h before the vasomotion studies. At each visit, blood samples were drawn for laboratory determinations of TG, total cholesterol (TC), HDL-cholesterol (HDL-C) and RLP-C concentrations before an oral fat load. Postprandial blood sampling was performed 4 h after the oral fat load, since previous studies in both healthy volunteers [12] as well as in patients with familial hypercholesterolaemia [11] have shown peak levels of both RLP-C and TG at this time point.

2.3. FMD assessment

The ultrasound measurements were performed in supine position at the elbow of the right arm using a vessel wall-movement system (Wall Track System, Pie Medical, Maastricht, The Netherlands), as described previously [8,9]. In short, an optimal two-dimensional B-mode image of the brachial artery was obtained. An M-line perpendicular to the vessel was selected. Next, the ultrasound system was switched to M-mode. The vessel-movement detector system repeatedly registered end-diastolic vessel diameter during a period of five to six cardiac cycles. This procedure was performed three times. The measurements were averaged to provide for a mean baseline diameter measurement.

By inflation of a blood pressure cuff for 4 min at a pressure of 100 mmHg above the systolic blood pressure, ischemia was applied to the forearm distal to the location of the transducer. Ultrasonography continued for 3 min after cuff release with measurements at 30-s intervals. The widest lumen diameter was taken as a measure for maximal diameter. Next, 0.4 mg sublingual nitroglycerine spray was administered as an endothelium-independent vasodilator. Nitroglycerine measurements were obtained at 2.5 and 5 min. Post fat-load FMD studies were performed 4 h after the oral fat load. The latter time point has been shown to correspond to the largest postprandial decrease in FMD response [10]. FMD and NTG were expressed as a percentage change relative to baseline diameter. Intra- and interobserver variability of this method at our institution is 3.6 and 4.1%, respectively.

2.4. Laboratory determinations

Plasma was obtained by centrifugation at 3000 rpm for 15 min at 4°C. TG and cholesterol were measured with a colorimetric assay (Monotest cholesterol kit no. 237574 and GPO-PAP no. 701912, Boehringer Mannheim, Germany). Coefficient of variance for TG and cholesterol was < 5%. Cholesterol was determined in the HDL fraction isolated by the heparin–MnCl₂ dextran-sulphate precipitation. Low-density lipoprotein-cholesterol (LDL-C) was calculated with the Friedewald formula.

2.4.1. Postprandial lipoprotein remnants as RLP-C

The RLP-C fraction was prepared using an immunoseparation technique described by Nakajima and co-workers [14,15]. Briefly, 5 μl of serum was added to 300 μl of mixed immunoaffinity gel suspension containing monoclonal anti-human apo-A-I (H-12) and anti-human Apo-B-100 (JI-H) antibodies (Japan Immunoresearch Laboratories, Takasaki, Japan). The reaction mixture was gently shaken for 120 min at room temperature followed by standing for 15 min. Next, 200 μl of the supernatant was
withdrawn for the assay of RLP-C. Cholesterol (coefficient of variance <3%) in the RLP fraction was measured by an enzymatic assay using a Cobas Mira S auto-analyzer (ABX Diagnostis, Montpellier, France).

2.5. Statistical analysis

Group values are expressed as mean±standard deviation. Differences in FMD between the three treatment periods and between baseline and post-fat loading were tested with a repeated measures ANOVA. Changes in lipid profile between treatment groups and before vs. after fat loading were also tested with repeated measures ANOVA. If variance ratios reached statistical significance, differences were analyzed with the Student–Newman–Keuls test for \( P < 0.05 \).

Pearson’s correlation or Spearman’s rank correlations were applied to evaluate relationships between parameters. A two-sided \( P \)-value of 0.05 was considered to be significant.

### 3. Results

The general characteristics of the study group at baseline during placebo, cerivastatin and gemfibrozil treatment are shown in Table 1. There were no statistically significant differences between baseline parameters, basal arterial diameter and basal FMD and NTG values.

The results of the lipid parameters, TG, TC, LDL-C and HDL-C, before as well as after the oral fat load and between the three treatment sessions are shown in Table 2. Upon fat loading, a significant increase in both TG and TC was observed in all treatment groups. Gemfibrozil treatment significantly lowered baseline TG concentration as well as baseline TC and LDL-C concentrations (Table 2). In contrast, cerivastatin treatment had no effect on the baseline TG concentration, whereas TC and LDL-C levels were lowered significantly (Table 2).

During placebo, fat loading induced an increase in TG, TC and RLP-C (Tables 2 and 3). During gemfibrozil, TG increase was significantly less compared to placebo therapy (\( P = 0.02 \); Table 2), whereas TC and RLP-C

### Table 1
General characteristics of the study group before an oral fat load

<table>
<thead>
<tr>
<th></th>
<th>Placebo</th>
<th>Gemfibrozil</th>
<th>Cerivastatin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Participants (n)</td>
<td>15</td>
<td>15</td>
<td>15</td>
</tr>
<tr>
<td>Age (years)</td>
<td>25.1 (4.4)</td>
<td>22.9 (2.6)</td>
<td>22.5 (2.8)</td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>23.1 (2.7)</td>
<td>22.9 (2.6)</td>
<td>22.5 (2.8)</td>
</tr>
<tr>
<td>Systolic blood pressure (mmHg)</td>
<td>135 (14)</td>
<td>133 (14)</td>
<td>137 (15)</td>
</tr>
<tr>
<td>Diastolic blood pressure (mmHg)</td>
<td>67 (9)</td>
<td>69 (9)</td>
<td>67 (9)</td>
</tr>
<tr>
<td>Vessel size (mm)</td>
<td>4.02 (0.68)</td>
<td>4.12 (0.54)</td>
<td>4.10 (0.49)</td>
</tr>
<tr>
<td>FMD (%)</td>
<td>9.1 (3.4)</td>
<td>9.0 (3.8)</td>
<td>7.9 (2.6)</td>
</tr>
<tr>
<td>NTG (%)</td>
<td>16.6 (5.9)</td>
<td>16.8 (5.5)</td>
<td>14.9 (4.0)</td>
</tr>
</tbody>
</table>

\( ^{a} \) Values are expressed as mean or percentages, with standard deviation in parenthesis.

### Table 2
Lipid profile before and 4 h after an oral fat load

<table>
<thead>
<tr>
<th></th>
<th>Placebo Preprandial</th>
<th>Placebo Postprandial</th>
<th>Gemfibrozil Preprandial</th>
<th>Gemfibrozil Postprandial</th>
<th>Cerivastatin Preprandial</th>
<th>Cerivastatin Postprandial</th>
</tr>
</thead>
<tbody>
<tr>
<td>Triglyceride (mmol/l)</td>
<td>1.1 (0.5)</td>
<td>2.0 (1.0)*</td>
<td>0.9 (0.4)**</td>
<td>1.4 (0.7)*</td>
<td>1.1 (0.7)</td>
<td>1.7 (1.0)*</td>
</tr>
<tr>
<td>Total cholesterol</td>
<td>4.7 (0.5)</td>
<td>4.9 (0.4)*</td>
<td>4.4 (0.8)**</td>
<td>4.6 (0.8)*</td>
<td>3.7 (0.6)**</td>
<td>3.8 (0.6)*</td>
</tr>
<tr>
<td>LDL cholesterol</td>
<td>3.0 (0.5)</td>
<td>2.8 (0.5)</td>
<td>2.7 (0.6)**</td>
<td>2.7 (0.7)</td>
<td>2.0 (0.5)**</td>
<td>1.8 (0.5)</td>
</tr>
<tr>
<td>HDL cholesterol</td>
<td>1.2 (0.3)</td>
<td>1.3 (0.3)</td>
<td>1.2 (0.3)</td>
<td>1.3 (0.3)</td>
<td>1.2 (0.4)</td>
<td>1.2 (0.4)</td>
</tr>
</tbody>
</table>

\( ^{a} \) Values are expressed as mean with standard deviation in parenthesis. \( * P < 0.05 \) vs. preprandial values; \( ** P < 0.05 \) vs. placebo values.

### Table 3
RLP-cholesterol before and 4 h after an oral fat load

<table>
<thead>
<tr>
<th></th>
<th>Placebo</th>
<th>Gemfibrozil</th>
<th>Cerivastatin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Preprandial RLP-C (mmol/l)</td>
<td>0.15 (0.06)</td>
<td>0.18 (0.07)</td>
<td>0.17 (0.09)</td>
</tr>
<tr>
<td>Postprandial RLP-C (mmol/l)</td>
<td>0.23 (0.07)*</td>
<td>0.23 (0.08)</td>
<td>0.19 (0.05)</td>
</tr>
<tr>
<td>(Post-pre)prandial RLP-C (mmol/l)</td>
<td>0.06 (0.04)</td>
<td>0.05 (0.09)</td>
<td>0.02 (0.07)**</td>
</tr>
</tbody>
</table>

\( ^{a} \) Values are expressed as mean with standard deviation in parenthesis. \( * P < 0.05 \) vs. preprandial values; \( ** P < 0.05 \) vs. placebo values.
increases were comparable to the placebo period (Tables 2 and 3). During cerivastatin, TG and TC increases upon fat loading were comparable to the increase during placebo therapy (Table 2). However, RLP-C levels did no longer show a significant increase upon fat loading (Table 3).

During placebo therapy an oral fat load induced an impairment in FMD, i.e. 9.1±3.4 to 4.3±3.3% (Fig. 1), whereas NTG remained unaltered, i.e. 16.6±5.9 to 14.1±4.1%. During gemfibrozil, the adverse effects of fat loading on FMD were comparable to the effects during the placebo period, i.e. 9.0±3.8 vs. 5.3±2.6% (Fig. 1). NTG response remained unaffected, i.e. 16.8±5.5 vs. 15.6±5.0%. During cerivastatin, the adverse effects of fat loading on FMD were completely abolished, i.e. 7.9±2.6 vs. 8.4±2.8% (Fig. 1). Again, NTG response remained unaffected, i.e. 14.9±5.7 vs. 14.7±4.4%.

No significant correlations could be demonstrated between changes in FMD and changes in lipid parameters. In particular, there was no significant correlation between RLP-C increase and FMD decrease postprandially during all treatment periods.

Of note, the results of the subject with the blood pressure elevation (systolic blood pressure 150 mmHg and diastolic blood pressure 85 mmHg) were comparable to the normotensive individuals. Since exclusion of the results did not significantly change the results, the data were included in the analysis.

4. Discussion

In the present study we demonstrate that cerivastatin, but not gemfibrozil prevents the impairment in FMD after a fat load. Concomitantly, cerivastatin abolishes the post-prandial increase in RLP-C, where the TG increase after a fat load is not significantly altered. In contrast, gemfibrozil clearly reduces the TG increase without significantly affecting the RLP-C increase after a fat load compared to placebo therapy. This combination of changes in ‘post-prandial’ lipid profile and FMD during these drugs lend further support to the concept that lowering of RLP-C, rather than lowering of total TG levels, may be responsible for the abolition of ‘postprandial’ endothelial dysfunction during statin use.

4.1. Fat loading and FMD

The anti-atherosclerotic actions of endothelium-derived nitric oxide (NO) are thought to be of major importance in preventing the development and progression of atherosclerosis [16]. Indeed, evidence has accumulated to show that peripheral endothelial dysfunction predicts the presence of coronary cardiovascular disease [17,18], whereas coronary endothelial dysfunction has recently been shown to have a clear predictive value for future cardiovascular incidents [5]. As a consequence, endothelial (dys)function is currently being evaluated as potential surrogate endpoint in cardiovascular prevention trials. In previous studies, we and others have repeatedly shown a significant and reproducible impairment in FMD upon ingestion of an oral fat load [8–10]. These observations may provide a rationale for the growing body of evidence from in vitro and in vivo studies showing that TRL, circulating predominantly in the postprandial period, are involved in the course of atherogenesis.

The postprandial plasma concentration of TRL comprises both intestinally derived chylomicrons and hepatic derived apoB-100-containing TRLs, as a result of reduced clearance of VLDL [19]. After entering the circulation, TRLs are hydrolysed by lipoprotein lipase (LPL) at the vascular endothelium, resulting in the formation of free fatty acids (FFA) and remnant particles. Evidence has accumulated to show that within the total TG fraction the RLPs, derived from exogenous (chylomicron remnants) and endogenous origin (β-VLDL and IDL), may be of pivotal importance for the adverse vascular effects. In support, a positive correlation was shown between RLP-C concentration and coronary endothelial dysfunction [7], whereas the correlation between postprandial total TG levels and endothelial dysfunction has provided heterogeneous results [8–10,20]. A lack of a direct relation between total TG levels and endothelial dysfunction is highlighted by Steinberg et al. [21], who failed to observe direct vascular effects of infusion of intralipid in spite of a two-fold rise in total TG levels. In contrast, administration of intralipid with concomitant heparin infusion, which activates vascular LPL resulting in the formation of FFA and remnant particles, was clearly associated with the onset of endothelial dysfunction [21]. The mechanisms responsible for the adverse vascular effects of RLP and/or
FFA remain to be elucidated. Recently, the importance of redox activity of RLP has been highlighted. Accordingly, in vitro and in vivo studies have shown that administration of antioxidants caused (partial) restoration of endothelial dysfunction induced by elevated RLP-C [3,22].

4.2. Effect of cerivastatin and gemfibrozil on lipid profile and FMD changes

In this study, postprandial RLP-C increase was significantly attenuated during cerivastatin therapy, whereas postprandial TG increase was lower but not significantly different from the increase in the placebo period. In this respect, statins are known to induce LDL-receptor gene expression, thereby increasing the number of LDL receptors resulting in subsequent inhibition of cholesterol synthesis [23]. Since the LDL receptor is also important for removal of remnant particles, statins can also improve RLP clearance, thereby influencing TG levels as a secondary effect. In addition, statins decrease hepatic VLDL secretion, resulting in less IDL/LDL-dense particles, whereas the catabolism of apo B-100 containing particles is increased. However, in the present study the latter mechanism seems to be of minor importance, since the alterations in postprandial lipid profile, i.e. RLP-C increase and lipolytically released FFA, are largely determined by the exogenous fat ingestion [24,25].

During fibrate therapy, the TG increase upon fat loading was significantly lower, whereas the rise in postprandial RLP-C was not significantly different from the placebo period. In this respect, fibrates primarily lower TG levels by influencing LPL and apoC-III gene expression [26]. Fibrates also decrease the availability of fatty acids for TG synthesis and may consequently influence VLDL secretion [26], thereby influencing cholesterol levels as a secondary effect. The combination of a relatively minor effect of gemfibrozil on RLP-C increase with a clear decrease in TG rise after a fat load implies that gemfibrozil is more effective in inducing lipolysis of TRLs than in inducing RLP-C removal from the circulation. Interestingly, the attenuated TG increase with unaffected RLP-C increase was accompanied by an impairment in FMD upon fat loading comparable to that during the placebo period.

The divergent effects of cerivastatin and gemfibrozil on vascular (dys)function, combined with the heterogeneous effects of these interventions on the lipid profile changes after an oral fat load, imply that the large buoyant TG containing particles are not of major importance for the adverse vascular effects observed upon fat loading. However, the attenuated RLP-C increase with concomitant prevention of ‘postprandial’ FMD impairment during cerivastatin lends further support to a potentially direct effect of RLP on endothelium dependent vascular responses. In contrast to Inoue [3], who demonstrated a positive correlation between (fasting) RLP-C and coronary endothelial dysfunction, we found no significant correlation between postprandial RLP-C levels and the degree of FMD impairment. However, this may relate to the smaller group size in the present study.

Besides lipid lowering effects, the statin-associated prevention of FMD impairment after a fat load could also be related to pleiotropic effects. These ‘direct’ effects of statins include (amongst others) attenuation of pro-inflammatory pathways (e.g. NFκB downregulation) and upregulation of anti-atherosclerotic mechanisms (e.g. upregulation of NO-synthase expression) [27–29]. However, since the baseline FMD during statin therapy was not significantly different from the placebo period, statin-induced modifications in the changes in ‘postprandial’ lipid profile seem to have a larger impact in the present study.

In conclusion, we show that cerivastatin therapy prevents the impairment in FMD after an oral fat load, whereas gemfibrozil has no protective effect. Taken together with the previously reported association of RLP-C and endothelial dysfunction, the association of beneficial effects on FMD and simultaneous abolition of ‘postprandial’ RLP-C increase during cerivastatin may suggest a role of RLP in postprandial FMD reduction.

Acknowledgements

Erik Stroes is a fellow of the Dutch Heart Foundation (NHS). We acknowledge Dr. T. Wang and Dr. K. Nakajima from Otusuka America Pharmaceutical, Inc. Rockville, Maryland, USA, for the disposal of the RLP-C assay. This study was supported by a research grant from Bayer AG.

References

angiotensin II type 1 receptor antagonism on postprandial endothelial function. J Am Coll Cardiol 1999;34:140–145.


