Regulatory Effects of Fenofibrate and Atorvastatin on Lipoprotein A-I and Lipoprotein A-I:A-II Kinetics in the Metabolic Syndrome

Dick C. Chan, PHD¹, Gerald F Watts, MD, DSC¹, Esther M.M Ooi, PHD¹, Kerry-Anne Rye, PHD², Juying Ji, PHD¹, Anthony G Johnson, MD³, P. Hugh R. Barrett, PHD¹.

1. Metabolic Research Centre, School of Medicine and Pharmacology, University of Western Australia, Perth, Western Australia
2. Lipid Research Group, The Heart Research Institute, Sydney, Australia; Department of Medicine, University of Sydney, Sydney, Australia
3. Bristol-Myers Squibb R & D, Princeton, NJ, USA

Short title: Regulation of HDL transport in metabolic syndrome

Address for correspondence:
PHR Barrett
E-mail: Hugh.Barrett@uwa.edu.au

Clinical trial no: NCT00632840, ClinicalTrials.gov

Submitted 16 March 2009 and accepted 24 July 2009.

This is an uncopiedited electronic version of an article accepted for publication in Diabetes Care. The American Diabetes Association, publisher of Diabetes Care, is not responsible for any errors or omissions in this version of the manuscript or any version derived from it by third parties. The definitive publisher-authenticated version will be available in a future issue of Diabetes Care in print and online at http://care.diabetesjournals.org.
**Objectives**- Subjects with the metabolic syndrome (MetS) have reduced HDL concentration and altered metabolism of HDL Lipoprotein (Lp) A-I and LpA-I:A-II particles. In MetS, fenofibrate and atorvastatin may have differential effects on HDL particle kinetics.

**Research Design and Methods**- Eleven men with MetS were studied in a randomized double-blinded crossover trial of 5-week intervention periods with placebo, fenofibrate (200mg/day) and atorvastatin (40mg/day). LpA-I and LpA-I:A-II kinetics were examined using stable isotopic techniques and compartmental modelling.

**Results**- Compared with placebo, fenofibrate significantly increased the production of both LpA-I:A-II (+30%, P<0.001) and apoA-II (+43%, P < 0.001), accounting for significant increases of their corresponding plasma concentrations (+10% and +23% respectively), but it did not alter LpA-I kinetics or concentration. Atorvastatin did not significantly alter HDL-cholesterol concentration or the kinetics of HDL particles.

**Conclusions**- In MetS, fenofibrate, but not atorvastatin, influences HDL metabolism by increasing the transport of LpA-I:A-II particles.
Atherogenic dyslipidaemia, reflected by elevated plasma triglyceride and reduced HDL concentrations, is a cardinal feature of the metabolic syndrome (MetS) (1). Recent findings from the Fenofibrate Intervention and Event Lowering in Diabetes (FIELD) study demonstrate that MetS subjects with atherogenic dyslipidaemia have the highest risk of cardiovascular disease (CVD) (2). Disturbed metabolism of HDL Lipoprotein (Lp) A-I and Lp-A-I:A-II particles may partly account for the increased risk of CVD (3).

In a previous study of eleven MetS subjects, we reported that fenofibrate, but not atorvastatin, had significant effects on HDL-apoA-I kinetics (4). Given the differential role of HDL LpA-I and LpA-I:A-II in reverse cholesterol transport (5), it is important to elucidate the precise effects of these agents on HDL particle kinetics. Using stored samples (4), we extend this study by investigating the effects of these agents on HDL LpA-I and LpA-I:A-II particle kinetics.

RESEARCH DESIGN AND METHODS

Subjects and study design. Eleven non-diabetic men with MetS entered a randomized, double-blind, placebo-controlled, cross-over trial which they were randomized to a 5-week treatment period of either fenofibrate (200 mg/day), atorvastatin (40 mg/day), or placebo. A two week washout phase was included at the end of each treatment period. All subjects provided written consent as approved by the Ethics Committee of the South Eastern Sydney Area Health Service. This clinical protocol including administration of [d$_3$]-leucine and blood sampling had been described previously (4).

Measurement of isotopic enrichments and calculation of kinetic parameters. HDL-apoA-I and apoA-II were isolated by ultracentrifugation and electrophoresis, delipidated, hydrolysed and derivatised as described (6). Isotopic enrichment was assessed using gas-chromatography mass-spectrometry with selected ion monitoring of derivatized samples. The SAAM II program (SAAM Institute, Seattle, WA) was used to fit the model to the observed tracer/tracee ratio data. The fractional catabolic rates (FCR) of apoA-I in Lp-A-I and Lp-A-I:A-II, apoA-I and apoA-II were derived from the model parameters giving the best fit. The corresponding production rates were calculated as the product of FCR and pool size.

Plasma Biochemistry. ApoA-I and apoA-II concentrations were determined by immunonephelometry (Dade Behring, Illinois, USA). ApoA-I concentration in Lp-A-I particles were measured by differential electroimmunoassay (Sebia, Moulmeaux, France). ApoA-I concentration in Lp-A-I:A-II particles was calculated as total apoA-I minus apoA-I in Lp-A-I. As previous reported (4), plasma lipid and glucose concentrations were determined by enzymatic methods. Plasma insulin was measured by radioimmunoassay. Insulin resistance was estimated using the homeostasis model assessment (HOMA) score.

Statistical analyses. Data at the end of the three treatment periods were compared using a mixed-effects model (SAS Proc Mixed, SAS Institute). To adjust for multiple comparison across the three treatment periods, we defined statistical significance at the 1.7% level.

RESULTS

The 11 subjects recruited were middle-aged, centrally obese, normotensive, dyslipidemic and insulin resistant. Compared with normolipidaemic lean subjects, MetS subjects exhibited hypercatabolism of both LpA-I and LpA-I:A-II with overproduction of LpA-I (data not shown).
As previously reported (4), fenofibrate significantly decreased plasma concentrations of triglyceride and apoB; it also significantly increased plasma HDL-\(_2\), HDL-\(_2\)- and HDL-\(_3\)-cholesterol concentration. Compared with placebo, atorvastatin significantly decreased total cholesterol, triglyceride, LDL-cholesterol and apoB concentrations. HOMA score did not change significantly on either treatment.

Table 1 gives the kinetic parameters for LpA-I, LpA-I:A-II, apoA-II and apoA-I after intervention. Compared with placebo, fenofibrate significantly increased the production rates of LpA-I:A-II by 30\% (\(P<0.001\)) and apoA-II by 43\% (\(P<0.001\)). Furthermore, fenofibrate increased the FCR of LpA-I:A-II (and apoA-II) by 16\% (\(P = 0.015\)) compared with placebo, accounting for the overall 10\% (\(P=0.005\)) increase in plasma HDL apoA-I FCR. Collectively, these kinetic effects accounted for the significant increase in concentration of LpA-I:A-II (10\%), apoA-II (23\%) and apoA-I (6\%) on fenofibrate treatment. Compared with placebo, atorvastatin did not significantly alter the kinetics or concentrations of LpA-I, LpA-I:A-II and apoA-II.

**CONCLUSIONS**

Our new findings show that in subjects with MetS, fenofibrate significantly increased the production of both LpA-I:A-II and apoA-II, accounting for the significant increase in their plasma concentrations. These effects were achieved with no significant alteration in insulin resistance and body weight. By contrast, atorvastatin had no significant effect on any parameters of HDL metabolism.

The fenofibrate data concur with previous reports showing that this agent increased the production of apoA-I in mixed hyperlipidaemia and MetS (7). We extend our previous study (4) by showing that the increased apoA-I production is restricted to apoA-I in LpA-I:A-II particles and is closely coupled to the increased production of apoA-II. This is consistent with the notion that the gene expression of both apoA-I and apoA-II are increased with this PPAR-\(\alpha\) agonist (8).

The lack of significant effect of atorvastatin on HDL-apoA-I kinetics concurs with a previous report (9). We extend these findings to MetS subjects and a wider range of HDL kinetic measurements including new data on apoA-II, LpA-I and LpA-I:A-II. We do not confirm data showing that atorvastatin increases LpA-I and decreases LpA-I:A-II concentrations in patients with coronary heart disease (10). This may be due to metabolic differences in study populations and that the CHD patients studied were not obese or insulin resistant. It is noteworthy that rosuvastatin, a more potent HDL-raising agent than atorvastatin, decreases LpA-I and LpA-I:A-II catabolism in subjects with MetS (11). A recent study by Verges et al also showed that rosuvastatin reduces HDL-apoA-I catabolism in type 2 diabetes (12). The precise reason for the difference between atorvastatin and rosuvastatin remains unclear.

Our kinetic findings could be clinically important. Decreased plasma LpA-I:A-II concentration is a predictor of coronary events in population studies (3), and in type 2 diabetes is independently associated with angiographic coronary disease (13). In the FIELD trial, fenofibrate altered HDL composition and increased the plasma concentration of A-II and LpA-I:A-II (14). Our study suggests that this may be due to increased production of apoA-II and LpA-I:A-II particles. The complementary effects of fenofibrate and atorvastatin on lipoprotein metabolism, including disparate changes in apoB-100 kinetics (4) and, as we show here, in the kinetics of HDL LpA-I and LpA-I:A-II particles supports the use of combination therapy to optimally regulate dyslipidaemia in MetS.
ACKNOWLEDGEMENTS

This study was funded by research grants from GlaxoSmithKline. PHRB is a National Health & Medical Research Council (NHMRC) Senior Research Fellow. DCC is a Career Development Fellow of the NHMRC. EMMO is a postdoctoral fellow of the National Heart Foundation of Australia. All authors have no relevant conflict of interest to disclose.
REFERENCES


# Regulation of HDL transport in metabolic syndrome

Table 1 Kinetic parameters of LpA-I, LpA-I:A-II, apoA-II and plasma apoA-I after treatment with fenofibrate, atorvastatin and placebo.

<table>
<thead>
<tr>
<th></th>
<th>Fenofibrate</th>
<th>Atorvastatin</th>
<th>Placebo</th>
<th>Differences (P-values)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(F)</td>
<td>(A)</td>
<td>(P)</td>
<td>F vs P</td>
</tr>
<tr>
<td>FCR, pools/day</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LpA-I</td>
<td>0.54±0.06</td>
<td>0.44±0.07</td>
<td>0.44±0.06</td>
<td>0.10±0.08 (0.186)</td>
</tr>
<tr>
<td>LpA-I:A-II</td>
<td>0.29±0.02</td>
<td>0.26±0.03</td>
<td>0.25±0.01</td>
<td>0.04±0.01 (0.015)</td>
</tr>
<tr>
<td>ApoA-II</td>
<td>0.29±0.02</td>
<td>0.26±0.03</td>
<td>0.25±0.01</td>
<td>0.04±0.01 (0.015)</td>
</tr>
<tr>
<td>ApoAI</td>
<td>0.33±0.02</td>
<td>0.29±0.03</td>
<td>0.29±0.01</td>
<td>0.04±0.02 (0.005)</td>
</tr>
<tr>
<td>Production rate, mg/kg/day</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LpA-I</td>
<td>6.41±0.62</td>
<td>5.50±1.08</td>
<td>6.14±1.16</td>
<td>0.27±1.03 (0.725)</td>
</tr>
<tr>
<td>LpA-I:A-II</td>
<td>11.90±1.10</td>
<td>9.18±0.76</td>
<td>9.18±0.67</td>
<td>2.72±0.63 (&lt;0.001)</td>
</tr>
<tr>
<td>ApoA-II</td>
<td>4.88±0.36</td>
<td>3.37±0.29</td>
<td>3.41±0.24</td>
<td>1.47±0.24 (&lt;0.001)</td>
</tr>
<tr>
<td>ApoAI</td>
<td>17.88±0.99</td>
<td>14.52±1.08</td>
<td>14.41±0.71</td>
<td>3.47±1.02 (0.002)</td>
</tr>
<tr>
<td>Plasma concentration, g/L</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ApoA-I in LpA-I</td>
<td>0.28±0.03</td>
<td>0.30±0.05</td>
<td>0.30±0.03</td>
<td>-0.01±0.02 (0.584)</td>
</tr>
<tr>
<td>ApoA-I in LpA-I:A-II</td>
<td>0.92±0.06</td>
<td>0.82±0.05</td>
<td>0.84±0.05</td>
<td>0.08±0.04 (0.016)</td>
</tr>
<tr>
<td>ApoA-II</td>
<td>0.38±0.02</td>
<td>0.29±0.01</td>
<td>0.31±0.01</td>
<td>0.07±0.01 (&lt;0.001)</td>
</tr>
<tr>
<td>ApoAI</td>
<td>1.20±0.06</td>
<td>1.11±0.04</td>
<td>1.13±0.05</td>
<td>0.07±0.03 (&lt;0.010)</td>
</tr>
</tbody>
</table>

Data are mean ± SEM; ApoA-I and apoA-II kinetics were determined from direct measurements of the corresponding tracer enrichment. The primary enrichment data for apoA-I and apoA-II were used to calculate LpA-I and LpA-I:A-II kinetic parameters.