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# Plasma apolipoprotein M responses to statin and fibrate administration in type 2 diabetes mellitus

**Authors**: Paul J.W.H. Kappelle[1,*], Josefin Ahnström[2,*], Bert D. Dikkeschei[3], Rindert de Vries[1], Wim J. Sluiter[1], Bruce H.R. Wolffenbuttel[1], Arie van Tol[4], Lars Bo Nielsen[5,6], Björn Dahlbäck[2], Robin P.F. Dullaart[1,**]

**Affiliations:**

[1]Department of Endocrinology, University Medical Center Groningen and University of Groningen, Groningen, The Netherlands.

[2]University of Lund, Department of Laboratory Medicine, University Hospital, Malmö, Sweden.

[3]Department of Clinical Chemistry, Isala Clinics, location Weezenlanden, Zwolle, The Netherlands.

[4]Department of Cell Biology & Genetics, Erasmus University Medical Center, Rotterdam, The Netherlands.

[5]Department of Clinical Biochemistry, Rigshospitalet, Copenhagen, Denmark

[6]Department of Biomedical Sciences, University of Copenhagen, Denmark.

*Contributed equally to the manuscript

** Corresponding author: R.P.F. Dullaart, MD, PhD, Department of Endocrinology, University Medical Center Groningen, PO Box 30001, 9700 RB Groningen, the Netherlands. E-mail: r.p.f.dullaart@int.umcg.nl

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**Abstract**

*Purpose:* Plasma apolipoprotein M (apoM) is potentially anti-atherogenic, and has been found to be associated positively with plasma total, LDL and HDL cholesterol in humans. ApoM may, therefore, be intricately related to cholesterol metabolism. Here, we determined whether plasma apoM is affected by statin or fibrate administration in patients with diabetes mellitus.

*Methods*: Fourteen type 2 diabetic patients participated in a placebo-controlled crossover study which included three 8-week treatment periods with simvastatin (40 mg daily), bezafibrate (400 mg daily), and their combination.

*Results*: ApoM was decreased by 7 % in response to simvastatin ($P< 0.05$ from baseline and placebo), and remained unchanged during bezafibrate and combined simvastatin+bezafibrate administration. Plasma apoM concentrations correlated positively with apoB-containing lipoprotein measures at baseline and during placebo ($P<0.02$ to $P<0.001$), but these relationships were lost during all lipid lowering treatment periods.

*Conclusions*: This study suggests that, even though plasma apoM is lowered by statins, apoM metabolism is to a considerable extent independent of statin- and fibrate-affected pathways involved in cholesterol homeostasis.

## 1. Introduction

Apolipoprotein (apo) M is a recently identified apolipoprotein which is mainly expressed in liver and kidney [1,2]. ApoM binds to lipoproteins via its retained signal peptide which serves as a lipophilic anchor [1]. ApoM is located mainly in the HDL fraction and to a minor extent in LDLs isolated from normolipidemic human plasma [3]. In human populations, plasma apoM levels are correlated positively with total cholesterol, LDL cholesterol and HDL cholesterol [4-8].

Interestingly, it has been shown that apoM retards atherosclerosis development in murine models [1,9,10]. Atheroprotective effects of apoM could possibly be attributed to the ability of this apolipoprotein to generate lipid poor pre-ß particles which serve as early acceptors of cellular cholesterol in the anti-atherogenic reverse cholesterol transport pathway, as well as to its anti-oxidative properties [1,3,7].

Experimental evidence is accumulating that apoM regulation may be intricately related to LDL cholesterol homeostasis. ApoM overexpression and apoM deficiency increase and decrease plasma cholesterol, respectively [10]. Recently, an increasing effect of apoM on VLDL+ LDL cholesterol was found to be dependent on an intact LDL receptor [11]. Moreover, plasma apoM is increased in murine models of LDL receptor deficiency [11]. These findings raise the possibility that plasma apoM levels may be decreased in response to administration of cholesterol lowering drugs. Nevertheless, the effects of pharmacological cholesterol lowering on human plasma apoM are still unexplored.

In this study we tested the extent to which statin and fibrate administration, alone and in combination, affect plasma apoM levels in type 2 diabetic patients.

## 2. Materials and methods

### *2.1. Subjects*

The medical ethics committee of the University Medical Center Groningen approved the study. Written informed consent was obtained from all subjects. Non-smoking male patients, aged > 18 years, with previously diagnosed type 2 diabetes mellitus participated. Diabetes treatment consisted of diet alone or combined with oral glucose lowering agents. Patients using lipid lowering drugs, insulin, thiazolidinediones and/or anti-hypertensive medication were excluded. None of the subjects had a cardiovascular disease history, hypertension (blood pressure > 140 mm Hg systolic and/or 90 mm Hg diastolic), severe hyperlipidemia (fasting plasma total cholesterol > 8.0 mmol/L and/or triglycerides > 4.5 mmol/L), thyroid disease, liver function abnormalities or elevated urinary albumin excretion (urinary albumin > 20 mg/L). Maximal alcohol allowance was 3 beverages daily. Participants were studied after an overnight fast on all occcasions. BMI was calculated as weight (kg) divided by height (m) squared. Blood pressure was measured after15 min. rest at the left arm in sitting position using a sphygmomanometer.

We performed a double blind, placebo-controlled cross-over study. Active medication and placebos were taken at 18.00 h. After baseline measurement, each patient first received either simvastatin 40 mg or bezafibrate 400 mg + matching

placebo daily for a period of 8 weeks, followed by a double placebo period of 8 weeks. This double placebo period was followed by alternative treatment with bezafibrate 400 mg daily or simvastatin 40 mg daily + matching placebo. Thereafter, participants received simvastatin 40 mg daily + bezafibrate 400 mg daily for another 8 weeks. Combined treatment with simvastatin + bezafibrate was given at the end of the study to avoid possible carry-over effects. Participants were instructed to continue their usual diet as well as their oral glucose lowering medication during the study.

*2.2 Laboratory methods*

Venous blood was collected into EDTA-containing tubes (1.5 mg/mL). Plasma samples were prepared by centrifugation at 1400 g for 15 min at 4 $^{0}$C. Glucose and glycated hemoglobin ($HbA_{1c}$) were measured shortly after blood collection. Samples for other assays were stored at -80 $^{o}$C until assay.

Plasma cholesterol and triglycerides were assayed by routine enzymatic methods (Roche/Hitachi cat nos 11876023 and 11875540 respectively, Roche Diagnostics GmbH, Mannheim, Germany). HDL cholesterol was measured using a homogeneous enzymatic colorimetric test (Roche/Hitachi cat. no. 03030024). Non-HDL cholesterol was calculated by subtracting HDL cholesterol from plasma total cholesterol. LDL cholesterol was calculated by the Friedewald formula. Apolipoprotein (apo) A-I and B were assayed by immunoturbidimetry (Roche/Cobas Integra Tina-quant cat no. 03032566 and 033032574, respectively, Roche Diagnostics). ApoM was assayed using a sandwich ELISA based on two highly specific monoclonal antibodies as described [4]. The procedure involves >1000 fold predilution of plasma samples

and the use of detergent buffer (Triton X-100) to avoid interference with lipids and other plasma components in the assay. The concentration of apoM in the calibrator was determined using a standard with known apoM concentration. The interassay coeffficient of variation (CV) was 4.9 %. Glucose was analyzed with an APEC glucose analyzer (APEC Inc., Danvers, MA). $HbA_{1c}$ was measured by high performance liquid chromatography (Bio-Rad, Veenendaal, The Netherlands; normal range 4.6-6.1%).

*2.3 Statistical analysis*

Data are given in mean±SD or median (interquartile range). Changes in variables were evaluated by two-way ANOVA. Duncan's test was applied to correct for multiple comparisons. Differences in relative changes in variables were tested by T-tests. Univariate relationships were calculated by Pearson correlation coefficients. Two-sided *P*-values <0.05 were taken as statistically significant.

## 3. Results

Fourteen type 2 diabetic patients (mean age 55±8 years, diabetes duration 4±2 years) participated. Their BMI, systolic blood pressure and diastolic blood pressure were 28.3±4.7 kg/$m^2$, 127±10 mm Hg and 80±7 mm Hg, respectively. Two patients were treated with diet only, 6 patients used metformin and 3 patients used sulfonylurea derivates alone; 3 patients used both drugs. These medications were continued during the study. BMI and blood pressure were unchanged during the study (data not shown). Fasting plasma glucose and $HbA_{1c}$ also remained unchanged (Table 1).

All (apo)lipoprotein measures were similar at baseline and during placebo administration (Table 1). Plasma total cholesterol and non-HDL cholesterol decreased during treatment with simvastatin, bezafibrate and their combination (Table 1). LDL cholesterol dropped during treatment with simvastatin alone and during simvastatin + bezafibrate. Plasma triglycerides decreased, whereas HDL cholesterol increased after bezafibrate and simvastatin + bezafibrate. Plasma apoB decreased during treatment with simvastatin alone and simvastatin + bezafibrate, whereas apoA-I increased during combined treatment (Table 1). The HDL cholesterol/non-HDL cholesterol ratio increased from 0.32±0.13 at baseline to 0.61±0.27 during combined treatment ($P$<0.001).

Plasma apoM decreased by 7 % during simvastatin monotherapy, but remained unchanged during bezafibrate and simvastatin + bezafibrate combined (Table 1). Baseline plasma apoM was correlated rather strongly and positively with baseline plasma total cholesterol, LDL cholesterol, non-HDL cholesterol and apoB (Table 2, Figure 1). These relationships were lost during all active treatment periods, but reappeared during placebo administration. In contrast, apoM was not significantly associated with HDL cholesterol and apoA-I at baseline, but a positive correlation of apoM with HDL cholesterol and apoA-I appeared during combination therapy (Table 2, Figure 1). Changes in apoM from baseline in response to simvastatin, bezafibrate and combination therapy were not positively correlated with changes in LDL cholesterol, non-HDL cholesterol and apoB during treatment ($P$>0.05 for all). Consequently, the LDL cholesterol, non-HDL cholesterol and apoB levels attained during administration of simvastatin, bezafibrate and simvastatin +

bezafibrate combined were still correlated with baseline apoM (r=0.574, *P*=0.032 to r=0.684, *P*=0.008, data not shown).

## 4. Discussion

This placebo-controlled study demonstrates that plasma apoM is decreased by simvastatin administration in type 2 diabetes. However, apoM remained unchanged during combined simvastatin and bezafibrate administration, contrasting expectedly large reductions in apoB-containing lipoproteins [12]. Our report thus shows that pharmacological lowering of apoB-containing lipoproteins does not decrease plasma apoM when obtained with an HDL-raising therapy.

We determined effects of statin and fibrate administration on apoM in type 2 diabetic patients in view of current recommendations that favour early lipid lowering drug intervention [13]. ApoM was found to be 9 % lower in type 2 diabetes, but this difference was attributable to diabetes-associated obesity [7]. Furthermore, statins are equally effective in cholesterol lowering in diabetic compared to non-diabetic subjects. Therefore, it is likely that the presently observed apoM response to statin treatment can be extrapolated to non-diabetic subjects.

Statins lower LDL cholesterol at least in part by increasing hepatic LDL catabolism via stimulation of LDL receptor-mediated lipoprotein uptake in response to cholesterol synthesis inhibition, and these effects are possibly accompanied by a decrease in apoB production [14,15]. We observed recently that complete absence of LDL-receptors in mice approximately doubles plasma apoM, and found that even HDL-associated apoM is affected by the LDL-receptor most likely in an apoE-dependent manner [11]. The modest lowering of apoM during simvastatin

monotherapy, as demonstrated in the present study, is consistent with the hypothesis that the LDL receptor also has some effect on plasma apoM levels in humans.

Fibrates decrease hepatic triglyceride synthesis, increase the catabolism and delipidation of large VLDL particles, and stimulate apoA-I synthesis [15]. In our study, plasma apoM was unaffected when bezafibrate was administered alone or in combination with simvastatin. As in an earlier report [6], plasma apoM before treatment was not significantly related to HDL cholesterol and apoA-I in type 2 diabetic subjects, suggesting that the diabetic state could weaken the strength of the relationship of HDL cholesterol with plasma apoM. Notably, apoM became correlated positively with HDL-cholesterol and apoA-I in response to combined fibrate + statin administration, which represented the treatment regimen that resulted in the highest HDL cholesterol/non-HDL cholesterol ratio. The responsible mechanism for this appearance of a relation between apoM and HDL cholesterol in response to treatment is unknown at present. Future experiments should address whether this phenomenon could involve a shift in apoM distribution between apoB-containing lipoproteins and HDL. ApoM exchanges rapidly between lipoproteins *in vivo* [11], and a shift in apoM lipoprotein distribution has been observed in murine models of LDL receptor and apoE deficiency [16]. Of further importance, apoM is decreased in apoA-I deficient mice [16]. Conversely, plasma apoM levels may predict apoA-I metabolism in obese individuals [8]. Altogether, it would seem possible that a decreasing effect of statins on total plasma apoM levels in association with LDL lowering is counteracted by an HDL and apoA-I increasing effect of fibrates.

Unlike animal studies which strongly suggest that apoM exerts anti-atherogenic properties [1,9,10], the relationship between apoM and cardiovascular disease in humans is unestablished at present [4,7,17]. It is, therefore, uncertain whether the small drop in plasma apoM in response to statin treatment would translate in cardiovascular risk modification. Of potential clinical relevance, higher apoB-containing lipoproteins levels during lipid lowering treatment were correlated with higher baseline apoM concentrations. Hence, future studies may reveal the validity of plasma apoM measurement in predicting the extent to which lipid targets are reached during pharmacological intervention.

In conclusion, regulation of human plasma apoM seems largely independent from statin and fibrate affected pathways involved in cholesterol homeostasis.

## Disclosure statement

The authors have nothing to disclose.

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## References

1. Nielsen LB, Christoffersen C, Ahnström J, Dahlbäck B. ApoM: gene regulation and effects on HDL metabolism. Trends Endocrinol Metab 2009;20:66-71.

2. Hu YW, Zheng L, Wang Q. Characteristics of apolipoprotein M and its relation to atherosclerosis and diabetes. Biochim Biophys Acta 2010;1801:100-5.

3. Christoffersen C, Nielsen LB, Axler O, Andersson A, Johnsen AH, Dahlbäck B. Isolation and characterization of human apolipoprotein M-containing lipoproteins. J Lipid Res 2006;47:1833-44.

4. Axler O, Ahnström J, Dahlbäck B. An ELISA for apolipoprotein M reveals a strong correlation to total cholesterol in human plasma. J Lipid Res 2007;48:1772-80.

5. Ahnstrom J, Axler O, Jauhiainen M, et al. Levels of apolipoprotein M are not associated with the risk of coronary heart disease in two independent case-control studies. J Lipid Res 2008;49:1912-7.

6. Plomgaard P, Dullaart RPF, de Vries R, Groen AK, Dahlbäck B, Nielsen LB. Apolipoprotein M predicts pre-ß-HDL formation: studies in type 2 diabetic and nondiabetic subjects. J Intern Med 2009;266:258-67.

7. Dullaart RPF, Plomgaard P, de Vries R, Dahlbäck B, Nielsen LB. Plasma apolipoprotein M is reduced in metabolic syndrome but does not predict intima media thickness. Clin Chim Acta 2009;406:129-33.

8. Ooi EM, Watts GF, Chan DC, et al. Association of apolipoprotein M with high-density lipoprotein kinetics in overweight-obese men. Atherosclerosis;210:326-30.

9. Wolfrum C, Poy MN, Stoffel M. Apolipoprotein M is required for prebeta-HDL formation and cholesterol efflux to HDL and protects against atherosclerosis. Nat Med 2005;11:418-22.

10. Christoffersen C, Jauhiainen M, Moser M, et al. Effect of apolipoprotein M on high density lipoprotein metabolism and atherosclerosis in low density lipoprotein receptor knock-out mice. J Biol Chem 2008;283:1839-47.

11. Christoffersen C, Pedersen TX, Gordts PL, Roebroek AJ, Dahlbäck B, Nielsen LB. Opposing effects of apolipoprotein M on catabolism of apolipoprotein B-containing lipoproteins and atherosclerosis. Circ Res 2010;106:1624-34.

12. Gavish D, Leibovitz E, Shapira I, Rubinstein A. Bezafibrate and simvastatin combination therapy for diabetic dyslipidaemia: efficacy and safety. J Intern Med 2000;247:563-9.

13. American Diabetes Association. Standards of Medical Care 2010. Diabetes Care 2010;33 (Suppl 1):S30-S32.

14. Ginsberg HN. Review: Efficacy and mechanisms of action of statins in the treatment of diabetic dyslipidemia. J Clin Endocrinol Metab 2006;91:383-92.

15. Bilz S, Wagner S, Schmitz M, Bedynek A, Keller U, Demant T. Effects of atorvastatin versus fenofibrate on apoB-100 and apoA-I kinetics in mixed hyperlipidemia. J Lipid Res 2004;45:174-85.

16. Faber K, Axler O, Dahlback B, Nielsen LB. Characterization of apoM in normal and genetically modified mice. J Lipid Res 2004;45:1272-8.

17. Ahnström J, Gottsäter A, Lindblad B, Dahlbäck B. Plasma concentrations of apolipoproteins A-I, B, and M in patients with critical limb ischemia. Clin Biochem. 2010;43:599-603.

**Table 1.** Fasting glucose, $HbA_{1c}$, plasma lipids, and apolipoproteins (apos) in 14 Type 2 diabetic patients at baseline and during administration of simvastatin (40 mg daily), bezafibrate (400 mg daily), placebo and simvastatin + bezafibrate combined.

| | **Baseline** | **Simvastatin 40 mg/day** | **Bezafibrate 400mg/day** | **Placebo** | **Simvastatin + Bezafibrate** | **ANOVA *P*-value** |
|---|---|---|---|---|---|---|
| **Glucose (mmol/L)** | 8.7 ±2.4 | 9.8±4.0 | 9.1±1.8 | 9.4±2.5 | 9.5±1.9 | 0.32 |
| **HbA1c (%)** | 7.5±1.3 | 7.7±1.8 | 7.1±1.2 | 7.1±0.9 | 7.1±0.) | 0.71 |
| **Total cholesterol (mmol/L)** | 5.10±0.84 | 3.50±0.74[a,b] | 4.45±0.64[a,c] | 4.90±0.67 | 3.71±0.64[a,b,d] | < 0.001 |
| **LDL cholesterol (mmol/L)** | 3.45±0.73 | 1.93±0.64[a,b] | 2.92±0.70[c] | 3.33±0.61 | 2.17±0.60[a,b] | < 0.001 |
| **Non-HDL cholesterol (mmol/L)** | 3.95±0.94 | 2.27±0.87[a,b] | 3.17±0.81[a] | 3.71±0.73 | 2.40±0.7[a,b,d] | < 0.001 |
| **HDL cholesterol (mmol/L)** | 1.15±0.34 | 1.23±0.38 | 1.27±0.35[a] | 1.18±0.32 | 1.31±0.38[a] | 0.011 |
| **Triglycerides (mmol/L)** | 1.78 (0.89-3.74) | 1.38 (0.85-2.05) | 1.00 (0.80-1.53)[a,b,c] | 1.66 (1.05-2.70) | 0.95 (0.70-1.73)[a,b,c] | < 0.001 |
| **ApoA-I (g/L)** | 1.26±0.19 | 1.30±0.22 | 1.32±0.20 | 1.27±0.18 | 1.38±0.24[a,b] | 0.016 |
| **ApoB (g/L)** | 1.05±0.26 | 0.67±0.23[a,b] | 0.91±0.24 | 1.01±0.21 | 0.71±0.19[a,b] | < 0.001 |
| **ApoM (g/L)** | 0.022±0.003 | 0.020±0.003[a,b,d] | 0.022±0.005 | 0.021±0.004 | 0.021±0.003 | 0.031 |

Data in mean±SD or in median (interquartile range). [a]$P < 0.05$ from baseline; [b]$P < 0.05$ from placebo; [c]$P < 0.05$ from simvastatin; [d]$P < 0.05$ from bezafibrate.

**Table 2.** Pearson correlation coefficients of apolipoprotein M with LDL cholesterol, non-HDL cholesterol and apolipoprotein B (apoB) in 14 type 2 diabetic patients at baseline and during administration of simvastatin (40 mg daily), bezafibrate (400 mg daily), placebo and simvastatin + bezafibrate combined.

| | **Baseline** | **Simvastatin** | **Bezafibrate** | **Placebo** | **Simvastatin+ bezafibrate** |
|---|---|---|---|---|---|
| **Total cholesterol** | 0.737** | 0.238 | 0.043 | 0.880*** | 0.234 |
| **LDL cholesterol** | 0.649* | 0.073 | -0.167 | 0.813*** | 0.212 |
| **Non-HDL cholesterol** | 0.680** | 0.095 | -0.165 | 0.814*** | 0.217 |
| **ApoB** | 0.687** | 0.206 | -0.190 | 0.770*** | 0.125 |
| **HDL cholesterol** | -0.054 | 0.247 | 0.461 | -0.410 | 0.669** |
| **ApoA-I** | 0.088 | 0.019 | 0.442 | -0.216 | 0.802*** |

*$P$<0.02; **$P$<0.01; ***$P$<0.001.

**Legends to Figure 1.** Relationships of plasma apolipoprotein M (apoM) with LDL cholesterol and HDL cholesterol at baseline (A,B) and during administration of simvastatin + bezafibrate combined (C,D).