A comparison of the effects of low- and high-dose atorvastatin on lipoprotein metabolism and inflammatory cytokines in type 2 diabetes

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Our objectives were to compare the effects of high and moderate-intensity atorvastatin treatment on lipoprotein metabolism and inflammatory markers and how frequently treatment goals are met in high-risk T2DM patients.

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Patients with T2DM and albuminuria (urinary albumin:creatinine ratio >5mg/mmol, total cholesterol <7mmol/l, proteinuria <2g/day, creatinine <200µmol/l) were randomised to receive atorvastatin 10mg (n=59) or 80mg (n=60) daily. Baseline and one-year follow-up data are reported.

Results:

Patients were at high cardiovascular disease (CVD) risk (observed combined mortality and non-fatal CVD annual event rate 4.8%). The non-high density lipoprotein cholesterol (HDL-C) goal of <2.6mmol/l was achieved in 72% of participants receiving high-dose atorvastatin, but only in 40% on low-dose (P<0.005). The proportion achieving apolipoprotein B (apoB) <0.8g/l on high and low-dose atorvastatin was 82% and 70% respectively (NS). Total cholesterol, triglycerides, low-density lipoprotein cholesterol (LDL-C), non-HDL-C, oxidised-LDL, apoB, glycapoB, apolipoprotein E and lipoprotein-associated phospholipase A2 decreased significantly, more so in participants on high-dose atorvastatin. Adiponectin increased and serum amyloid A (SAA) decreased without dose dependency. Neither dose produced significant changes in HDL-C,

cholesterol efflux, high-sensitivity C-reactive protein, glycated haemoglobin, serum paraoxonase-1, lecithin:cholesterol acyl transferase or cholesteryl ester transfer protein.

Conclusions:

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Highlights

- High-dose atorvastatin is more effective in achieving non-HDL-C targets.
- High-intensity treatment produced greater decreases in LDL-related parameters.
- Neither dose produced significant changes in markers of HDL functionality.
- A lower apoB therapeutic target of $<0.7\text{g/l}$ is proposed.

**A comparison of the effects of low and high dose atorvastatin on
lipoprotein metabolism and inflammatory cytokines in type 2 diabetes:
results from the Protection Against Nephropathy in Diabetes with
Atorvastatin (PANDA) randomised trial**

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KEY WORDS

Apolipoprotein B, atorvastatin, glycated apolipoprotein B, high-density lipoprotein, inflammatory cytokines, low-density lipoprotein, therapeutic targets, type 2 diabetes mellitus, very-low-density lipoprotein.

ABBREVIATIONS

Apolipoprotein	apo
Apolipoprotein B100	apoB
Cardiovascular disease	CVD
Cholesterol Treatment Trialists'	CTT
-Cholesterol	-C
Collaborative Atorvastatin Diabetes Study	CARDS
C-reactive protein	CRP
High density lipoprotein cholesterol	HDL-C
Lipoprotein associated	Lp-
Low density lipoprotein cholesterol	LDL-C
Modification of diet in renal disease	MDRD
National Institute of Health and Clinical Excellence	NICE
Paraoxonase 1	PON1
Phospholipase A2	PLA2
Protection Against Nephropathy in Diabetes with Atorvastatin	PANDA
Serum amyloid A	SAA
Small-dense LDL	SD-LDL
Triglyceride	TG
Type 2 diabetes mellitus	T2DM
Urinary albumin:creatinine ratio	ACR
Very low density lipoprotein cholesterol	VLDL-C

INTRODUCTION

Statin treatment was recommended in type 2 diabetes mellitus (T2DM) following publication of the Collaborative Atorvastatin Diabetes Study (CARDS) ¹, the first trial of a statin in primary prevention of atherosclerotic cardiovascular disease (CVD) in T2DM, and the Cholesterol Treatment Trialists' (CTT) collaboration meta-analysis combining CARDS results with those of T2DM participants, who had been included in earlier primary and secondary prevention trials not designed specifically to test that a statin could decrease CVD incidence in T2DM ². Initially there was concern expressed about whether this recommendation should apply universally to T2DM without clinical evidence of CVD ³. However, recently even in people without diabetes, the threshold for 10-year absolute CVD risk required for the introduction of statin treatment has been decreased to 10% in the UK ⁴ and 7.5% in the USA ⁵. There must be few T2DM patients whose CVD risk does not exceed these thresholds and thus the debate has moved on to when moderate intensity statin therapy, such as atorvastatin 10mg daily, and when intensive statin treatment, such as atorvastatin 80mg daily, is warranted. Certainly, the more intensive approach is justified in secondary prevention and may also be in a proportion of patients who have yet to experience a vascular event, but are assessed as being at particularly high risk on other grounds, such as duration of diabetes, nephropathy and hypertension ⁶. There is a dichotomy of views about the extent to which treatment should be targeted at achieving specific goals in terms of either low density lipoprotein cholesterol (LDL-C) or non-high density lipoprotein cholesterol (non-HDL-C). The National Lipid Association ⁷ and the European Society of Cardiology and allied societies ⁸ have retained the goals of LDL-C <2.6mmol/l and <1.8mmol/l depending on the degree of CVD risk whereas the American College of Cardiology/ American Heart Association ⁵ and the National Institute of Health and Clinical Excellence (NICE) ⁴ have less emphasis on goals, although NICE recently revised its guidance by advising at least a 40% decrease in non-HDL-C on follow-up ⁴. This is frequently impractical, because the pre-treatment value is unknown when, for example patients have already commenced statin treatment by the time they are referred to a hospital diabetes clinic or when they present with an acute coronary syndrome. Surprisingly too, in diabetes, with the exception of their effect on LDL-C there is a dearth of information about the effects of intensive statin treatment as opposed to moderate statin treatment on other potentially atherogenic aspects of lipoprotein metabolism. Even for LDL-C the proportion of patients in whom therapeutic targets are achieved is largely unreported in diabetes. Furthermore, the effects of intensifying statin treatment on inflammatory cytokines implicated in atherosclerosis are unknown. We have compared the effects of atorvastatin 10mg with those of 80mg daily in participants in the first year of the Protection Against Nephropathy in Diabetes with Atorvastatin (PANDA) trial ⁹.

An atherogenic lipoprotein profile consisting of low HDL-C, raised triglycerides and increased small-dense LDL (SD-LDL) particles is common in T2DM ^{10,11}. SD-LDL particles are more susceptible to oxidation and glycation, possibly because their plasma half-life is increased compared with more buoyant LDL particles and more apolipoprotein B100 (apoB) lysine groups are exposed on their surface ¹². Glycooxidation increases LDL atherogenicity and in T2DM there may be accelerated LDL glycooxidation ¹³. Phospholipase A2 (PLA2; also known as platelet activating factor) largely located on LDL is associated with predisposition to atherosclerosis ¹³. Apolipoprotein E (apoE) may also be involved in atherogenesis in diabetes, which, like familial dysbetalipoproteinaemia in which apo E is clearly raised, is associated with peripheral atherosclerosis. Apo E is cleared through hepatic LDL receptors upregulated by statins, but again no information is available about dose dependency ¹⁴. Atorvastatin has a modest effect on HDL-C (1) and size ¹⁵ in patients with T2DM, but less is known about its effect on HDL functionality, which is impaired in T2DM ^{16,17}. Two important aspects of HDL functionality are its capacity to decrease oxidative modification of LDL, in which paraoxonase 1 (PON1) and other HDL components participate ¹⁸, and its involvement in the removal of excess cellular cholesterol (cholesterol efflux capacity). The functionality of HDL is impaired in inflammatory states when serum amyloid A (SAA), an acute phase reactant component of HDL, increases ^{19,20}. Inflammation contributes importantly to atherosclerosis ²¹ and, although statins are known to influence certain inflammatory markers ²², the extent to which this is dependent on dose is largely unknown. Decreased adiponectin in T2DM and metabolic syndrome ²³ has been linked to increased C-reactive protein (CRP) ²⁴. The degree to which adiponectin levels decline is associated with the severity of dyslipidaemia in diabetes ²⁵. Some statins may increase adiponectin ²⁶. However, little is known about the relative effects of more intensive regimens. This study compares the effect of low and high-dose atorvastatin (increasingly recommended in diabetes) on LDL and LDL-related CVD risk factors, systemic inflammation and HDL functionality and the achievement of lipoprotein targets in patients with T2DM and optimally-managed early renal disease, who took part in the PANDA study ⁹.

MATERIALS AND METHODS

Study population

The PANDA study was a double-blind randomised clinical trial comparing the effects of atorvastatin 10 mg/day (N=59) vs 80 mg/day (N=60) on renal endpoints in T2DM patients with microalbuminuria or proteinuria recruited in Manchester, UK ⁹. The study was approved by the local ethics committee and the reported investigations were carried out in accordance with the principles of the Declaration of Helsinki as revised in 2000. Details of intervention, randomisation, sampling and primary and secondary end-point outcomes have been described previously ⁹. Briefly, inclusion criteria were: age

>40 years, T2DM, as defined by the WHO ²⁷, and a urinary albumin:creatinine ratio (ACR) >5 mg/mmol on two occasions. Exclusion criteria were: pregnancy or potential pregnancy, urinary protein excretion >2g daily, serum creatinine >200µmol/l, blood pressure >160/>90mmHg at randomisation, serum cholesterol >7mmol/l and fasting serum triglycerides ≥6 mmol/l, hepatic transaminase >2x normal, alkaline phosphatase >1.5x normal, HbA1c > 10% (85.8 mmol/mol), untreated hypothyroidism, intolerance of angiotensin II receptor-blocking drugs or statins, taking atorvastatin >10mg daily (or the equivalent effective dose of another statin) or use of any other lipid lowering medication, illness other than diabetes and its complications likely to influence the trial outcome. All patient visits were scheduled in the morning following an overnight fast from 10.00 pm the previous evening. Participants attended scheduled visits after randomisation at 3 months, 6 months and then every 6 months. We included baseline and 12 month samples in this study.

Laboratory Methods

The laboratory participated in the UK National External Quality Assessment Service (UKNEQAS, Birmingham, UK) for quality control of general blood chemistry and urinary chemistry; and in the Wales External Quality Assurance Scheme (WEQAS, Cardiff, UK) for blood lipid profile, apolipoprotein B and apolipoprotein AI. HbA1c was analyzed using a Roche Modular P unit (Roche Diagnostics, Burgess Hill, UK). Serum liver function tests, electrolytes, fasting glucose calcium, phosphate and the full blood count were also analyzed using routine automated methods. Cholesterol was measured using the CHOD-PAP method, triglycerides by the GPO-PAP method and apolipoproteins AI (apoAI) and apoB (ABX Diagnostics, Shefford, UK) were assayed using immunoturbidimetric assays with a Cobas Mira analyzer (Horiba ABX Diagnostics, Nottingham, UK) with calibration traceable to International Federation of Clinical Chemistry primary standards ²⁸ to ensure comparability with other published studies. Serum and urinary creatinine were assayed individually using the rate blanked compensated Jaffe method using an Hitachi 747 analyzer (Roche Diagnostics, Indianapolis). Serum HDL-C was assayed using a second generation homogenous direct method (Roche Diagnostics, Burgess Hill, UK). The laboratory participated in the RIQAS (Randox Laboratories, Dublin, Ireland) scheme which is CDC calibrated. Non-HDL-C was determined by subtraction of HDL-C from total serum cholesterol and LDL-C by the Friedewald equation²⁹. In 6 participants whose baseline fasting triglyceride level exceeded 4.5mmol/l, VLDL-C and HDL-C were subtracted from total serum cholesterol to obtain the LDL-C. Ultracentrifugation of 1 ml volumes of plasma adjusted to the desired density (D) in polycarbonate tubes with the Beckman Optima TLX ultracentrifuge for 4h at 435,680xg in a fixed angle TLA120.2 rotor (Beckman Coulter, High Wycombe, UK) was used to measure VLDL-C (D1.006g/ml supernatant) and SD-LDL- apoB (D1.044g/ml infranant) ³⁰. PON1 activity was measured in serum samples using paraoxon as substrate ³¹. Total glycated apoB (glyc apoB) was assessed by ELISA kits from Glycor, Exocell Inc, USA with an intra- and inter-assay coefficient of variance of 3.5 and 14.9%. Oxidised LDL (OxLDL)

was assessed by ELISA kits from Mercodia, Sweden with an intra- and inter-assay CV of 5.8% and 4.6% respectively. SAA was assayed by ELISA using kits from Invitrogen Ltd, Paisley, UK. The detection limit for SAA was <4ng/ml with intra- and inter-assay CV of 6.1 and 7.4 %, respectively. Lipoprotein-associated-PLA2 (Lp-PLA2) mass was determined using the diaDexus PLAC ELISA (diaDexus Inc, South San Francisco, USA). The detection limit for Lp-PLA2 was <0.4 ng/ml with intra- and interassay CV of 6.2% and 6.7%, respectively. High-sensitivity CRP (hs-CRP) was measured in serum by an in-house, antibody sandwich ELISA technique using anti-human CRP antibodies, calibrators and controls from Abcam (Cambridge, UK). Total multimeric adiponectin concentration in plasma was determined using ELISA kits from ALPCO Diagnostics, Salem, New Hampshire, USA through Diagenics Ltd, Milton Keynes, UK. The sensitivity is defined by an absorbance of not less than 0.9 OD at the highest concentration in the assay (range 0.075 – 4.8ng/ml) and the intra-assay CV is <15%. Lecithin cholesterol ester transferase (LCAT) and cholesteryl ester transfer protein (CETP) activities were measured using our modification of the Stokke Norum method using the patient's own lipoproteins as substrates ³².

The cholesterol efflux capacity of HDL was determined in an assay that has been validated previously ³³. In summary J774A.1 cells were incubated with radiolabelled cholesterol. These cells were then incubated with apoB-depleted serum for 4 hours. After incubation, the cell media were collected and cells were washed with phosphate buffered saline (PBS) and dissolved in 0.5ml 0.2N NaOH to determine radioactivity. Cellular cholesterol efflux was expressed as the percentage of radioactivity in the medium from the radioactivity in the cells+medium. Cholesterol efflux was calculated using the following formula:

$$\text{Cholesterol efflux (\%)} = \frac{\text{radioactivity in medium}}{\text{radioactivity in cell} + \text{radioactivity in medium}} \times 100$$

To calculate cholesterol efflux at baseline and after treatment, we subtracted efflux to serum-free serum media (control).

Study Design and Statistical Analysis

The primary purpose of the study was to compare findings in participants randomly allocated to atorvastatin 10mg and 80mg daily after 12 months of treatment. A prespecified linear mixed model for longitudinal data was used to assess differences in biomarker levels at 12 months after randomisation between high- and low-dose atorvastatin groups ³⁴. The model adjusted for baseline levels of biomarkers, age, sex, baseline MDRD eGFR (modification of diet in renal disease study estimated GFR), baseline total cholesterol, baseline HbA1c and use of washout period. This per protocol adjustment did not change the statistical significance of our findings. Where mean differences are stated, multivariable 95% CI are provided. In order to establish whether atorvastatin

in either dose or in all participants irrespective of dose was associated with alterations in the study parameters, secondary (post hoc) analyses of unadjusted within-study group changes in variables between baseline and 12 months were assessed using Student's t test or the Wilcoxon test depending on the data distribution. Results with a Gaussian distribution are reported as mean (SD) and as median (interquartile range), if skewed. The percentages attaining target lipoprotein levels are presented with 95% CI's. All analyses were done using Stata (release 10 StataCorp College Station, Texas USA). Although some variables were not strictly independent and were, for example, components of LDL or HDL, to allow for multiple testing and to interpret results conservatively, P values >0.025 were considered non-significant, in the range 0.025 to 0.002 they were considered statistically significant with caution and <0.002 definitely significant ³⁵.

RESULTS

Demographics

Demographic data are summarised in table 1. Successful randomisation of patients to low- and high-dose atorvastatin groups was indicated by the lack of any significant differences between demographic characteristics and laboratory results at baseline (tables 1 and 2). Baseline results did not differ significantly between 10 and 80 mg dose groups. There were 12 fatal or non-fatal CVD events (coronary heart disease or cerebral infarction). This event rate (4.8% per year) did not differ significantly between treatment groups.

Dose-dependent differences after 12 months of treatment

The primary analysis was to test for dose-dependent differences at 12 months. Twelve-month data and multivariable-adjusted mean (95%) differences between high- and low-dose atorvastatin groups are shown in table 3. Total cholesterol, TG, LDL-C, non-HDL-C, apoB, glycapoB, oxLDL, Lp-PLA2 and apoE were significantly lower on atorvastatin 80 mg compared to 10 mg daily (table 3). The incremental decrease in total serum TG on 80mg daily was of borderline significance (P<0.022).

Atorvastatin-induced changes from baseline

In secondary analyses the direction and extent of the effects of the 10mg and 80mg daily regimens was assessed and the two doses compared by the percentage change in biomarkers between baseline and 1 year. The importance of these analyses was firstly that it established which variables were affected by the lower as well as the higher dose of atorvastatin and secondly whether any lack of dose-dependency at 12 months was the result of non-response to either dose or a similar response to both doses. Thus, although no dose-dependent differences in SD-LDL apoB or adiponectin were

evident at 12 months, there was a significant decrease in SD-LDL apoB and a significant increase in adiponectin on atorvastatin regardless of dose (table 4 and figure 1). Serum apoE decreased significantly only in those who received high dose atorvastatin. Serum hsCRP and SAA showed great variability in their response to atorvastatin with either dose. The lower apoA1 value on 80mg as opposed to 10mg daily (statistically non-significant) was due more to a small increase on 10mg daily than a decrease from baseline.

A pooled analysis of both doses of atorvastatin confirmed that the drug had no statistically significant effect on LCAT, CETP, PON1, hsCRP, HbA_{1c} or cholesterol efflux (table 4). That atorvastatin increases adiponectin and decreases SAA was, however, clear from this larger dataset ($p < 0.01$). GlycapoB correlated with total serum apoB both before ($r = 0.43$; $P < 0.001$) and after atorvastatin treatment ($r = 0.48$; $P < 0.001$) whereas there was no correlation with HbA_{1c}. The change in glycapoB correlated with change in apoB ($r = 0.30$; $P < 0.01$) and with change in apoE ($r = 0.31$; $P < 0.01$). Serum apoB correlated with Lp-PLA₂ both at baseline ($r = 0.26$; $P < 0.01$) and 12 months ($r = 0.47$; $P < 0.0001$).

Magnitude of lipoprotein changes and achievement of therapeutic targets

On both doses, the magnitude of the changes was LDL-C > non-HDL-C > apoB. On atorvastatin 10mg daily the changes were 32.2%, 26.7% and 26.2% respectively and on 80mg daily they were 49.7%, 43.8% and 38.1%.

The LDL-C goal of statin treatment for high risk patients (< 1.8 mmol/l) was achieved by only 29% of participants on atorvastatin 10mg daily but by 68% on 80mg (table 5). The corresponding non-HDL-C target (< 2.6 mmol/l) was attained by 40% of patients treated with atorvastatin 10mg daily and by 72% on 80mg. For both the LDL-C and non-HDL-C targets the proportion of people reaching goal was significantly greater on 80mg daily. There was no significant difference between the two doses in attaining the apoB target (< 0.8 g/l) which was met by 70% receiving 10mg (significantly greater than LDL-C and non-HDL-C; $P < 0.05$) and by 82% on 80mg daily. We also therefore tested the proportion attaining a lower apoB target of < 0.7 g/l. On both doses, this was reached by a similar proportion of participants to the LDL-C and non-HDL-C targets (Table 5).

DISCUSSION

We compared the effects of atorvastatin 10mg with 80mg daily after one year in high-risk patients with T2DM. The primary aim of statin treatment is to lower the circulating concentration of apoB-containing lipoproteins. In the present trial, wherein pre-treatment LDL-C levels just exceeded 3 mmol/l, the overall decrease with atorvastatin 80mg daily was 1.6 mmol/l, representing an additional reduction of 0.5 mmol/l compared to the lower dose. This is consistent with the typical statin dose

response in which each doubling of the dose results in an additional 6% decrease in LDL-C³⁶, which would predict an extra 17% reduction from an increase in dose from 10 to 80mg daily. Participants with T2DM in the CTT collaboration meta-analysis of randomized statin trials had the same decrease in relative CVD risk as those without diabetes, amounting to 22% for each 1mmol/l decrease in LDL-C³⁷. It would be anticipated therefore that the 1.6mmol/l reduction in LDL cholesterol on atorvastatin 10 mg daily would produce a 33% reduction in CVD events and the 2.1mmol/l decrease on 80mg a 41% reduction^{37,38}. These predictions are close to the 37% decrease in CVD incidence we reported in CARDS¹. PANDA was not powered to confirm this, but rather to examine in detail the effects of two doses of atorvastatin on lipoprotein metabolism and inflammatory markers.

Some recent recommendations for lipid management in diabetes emphasise the importance of achieving therapeutic targets for either LDL-C, non-HDL-C or apoB (the major component of the protein moiety of both LDL and VLDL)^{7,8,39}. In our high-risk patients, the goal of treatment recommended is to lower LDL-C to <1.8mmol/l, non-HDL-C to <2.6mmol/l or apoB to <0.8g/l. Our investigation reveals that, 29% and 40% of patients achieved their LDL-C and non-HDL-C targets respectively on atorvastatin 10mg daily, whereas 68% and 72% did on 80mg daily ($P<0.005$) (table 5). The apoB target was achieved in 70% of patients receiving 10mg daily. This suggests that the apoB target, which was intended to be equivalent to the LDL-C goal of 1.8mmol/l, is too high. We previously reported that apoB is a better treatment target than calculated LDL and non-HDL-C in statin-treated patients⁴⁰ and earlier results from CARDS indicated that a lower apoB target of perhaps <0.7g/l would more closely approximate to an LDL-C of 1.8mmol/l⁴¹. Sniderman and colleagues have reached a similar conclusion from a systematic review of population studies⁴². The present results in high risk people with diabetes independently reinforce this view at two intensities of statin treatment.

ApoB-containing lipoproteins may vary in their atherogenicity. SD-LDL, oxidatively modified LDL and glycapoB^{11,43,44} have all been found to participate more readily in potentially atherogenic processes, such as monocyte/macrophage foam cell formation^{11,12,43,44}. In PANDA, glycapoB showed significant additional reduction on 80mg daily (figure 1 and table 3). This is interesting in view of the debate about the effect of statins on glycaemia⁴⁵⁻⁴⁷. In the present study, neither atorvastatin dose had a statistically significant effect on HbA1c or fasting glucose, possibly because any effect is too small for it to be discernible in 119 participants as opposed to the larger CARDS where increases were reported in the first year of treatment⁴⁷. Our finding that a highly significant decrease in glycapoB occurs with statin therapy may have some bearing on how, in CARDS and other studies, statins continue to protect against atherosclerotic complications in a dose-dependent manner despite a slight deterioration in glycaemia⁴⁷. In the present study glycapoB correlated with total serum apoB both before ($r=0.43$; $P<0.001$) and after atorvastatin treatment ($r=0.483$; $P<0.001$) whereas there was no correlation with HbA1c. Thus serum apoB may be more important in determining the concentration of

glycapoB than HbA_{1c} as we have previously reported⁴⁸. In our study, Lp-PLA₂ correlated with both serum apoB and SD-LDL and was decreased in a dose-dependent manner by atorvastatin. A similar phenomenon has been reported in type 1 diabetes⁴⁹, but increasingly, although still regarded as a risk marker⁵⁰, the causal link between Lp-PLA₂ and atherosclerotic CVD is being questioned⁵¹.

Both doses of atorvastatin lowered triglycerides, but the higher dose did not do so more significantly than the lower dose. The triglyceride-lowering effect of statins in general is largely achieved at doses lower than are required to achieve their greatest LDL-lowering effect and our present finding is consistent with a meta-analysis of the triglyceride statin dose-response⁵². Neither dose of atorvastatin significantly altered HDL-C in our study. This is consistent with previous reports^{1,53-55}. A trend for apoAI to rise on 10mg daily was abolished at 80mg daily. We recently reported results of a meta-analysis examining whether CVD was more effectively prevented by statins which raise circulating levels of HDL⁵⁴. There was no evidence that CVD incidence was reduced by statin-induced increases in HDL-C and only a small possible benefit from the effect of some statins in raising apoAI. The present results did not reveal the decrease in CETP activity which is evident with, for example, rosuvastatin with its greater HDL-raising capacity^{55,56}. This may be relevant to the current debate about whether raising HDL-C by CETP inhibition is likely to prove beneficial. There was also no increase in the antioxidative enzyme, PON1 activity, predominantly located on HDL¹⁸, on either dose of atorvastatin. A recent meta-analysis indicates that statins may increase PON1, but the effect is small and the development of alternative strategies to raise PON1 remains potentially important⁵⁷. Consistent too with the lack of change in HDL components is the absence of any effect of atorvastatin on cholesterol efflux, although there is currently controversy about whether HDL is the limiting factor in this process⁵⁸.

We report that atorvastatin raised adiponectin levels irrespective of dose. This is consistent with the findings of a recent meta-analysis⁵⁹. Adiponectin produced by adipose tissue is diminished in T2DM and, because of the positive association of adiponectin with HDL, this has been linked to a more favourable lipoprotein profile²⁵. However, the atorvastatin-induced increase in the present PANDA study was unaccompanied by a significant effect on HDL functionality in terms of PON1 activity or cholesterol efflux. CRP is the most widely investigated inflammatory factor of potential importance in CVD, yet its causal involvement remains uncertain⁶⁰. In the current study neither atorvastatin dose lowered hsCRP consistently, although the 80mg dose was associated with an apparent reduction, which did not achieve statistical significance. It is possible that a further trial with repeated measurement to overcome its considerable biological variation would be necessary to explore any statin effect. The lack of change from baseline in PANDA in atorvastatin-treated participants is entirely consistent with the similar findings in the active treatment arm of CARDS⁶¹, but in that study an increase in hsCRP occurred on placebo, which gave rise to a higher level compared to active

treatment. PANDA was a dose comparison study and was not designed to enquire as to whether atorvastatin can diminish CRP rises which might spontaneously occur in untreated people.

Our study has several strengths: we used data from a randomised controlled trial in which baseline characteristics and biomarkers were well-matched between treatment groups; we studied several established and several novel biomarkers; our external validity is likely to be high because we studied high-risk patients with T2DM and albuminuria that are likely to be representative of patients in other settings; we provided robust data on between-group comparisons by adjusting for residual differences in baseline characteristics; we had a moderately long one-year follow-up. We acknowledge some limitations. The trial was conducted in patients with mild to moderate diabetic nephropathy, but in that regard, they were probably not dissimilar to many commonly encountered T2DM patients: they did not have the elevated LDL-C levels which frequently characterise those with more severe renal dysfunction. The primary objective of our present report was to compare intensive and less intensive atorvastatin after one year of treatment. The longitudinal comparisons with baseline levels are secondary analyses, although the randomisation would seem to have reduced the likelihood that this produced bias and the consistency of the primary and secondary analyses would also support this conclusion. The trial design permitted a more detailed exploration of statin effects in T2DM than has hitherto been possible and it was large enough not to miss effects of a magnitude likely to have clinical relevance.

In conclusion atorvastatin at higher dose in T2DM produces additional decreases in all parameters relating to LDL, particularly glycaPoB. Furthermore, potentially favourable effects in certain inflammatory cytokines occurred. There was no evidence for effects on HDL functionality. Profiling of the effects of lipid-modifying drugs beyond their LDL-C lowering capacity may be important in identifying why some will emerge as more successful in combatting CVD.

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DECLARATION

MF, MG, MJG, PND, SA, JHH, SK, TS and YL have no conflict of interest to declare. HS received research grants from Synageva, Pfizer, Amgen and MSD and honoraria from Sanofi, Synageva, BMS, Lilly, AstraZeneca, Pfizer, Takeda, AMGEN and MSD. MKR received research support and honoraria from Lilly and GSK and funding to attend meetings from NovoNordisk and MSD. NY received research grants from Sanofi and honoraria from Janssen, Astra-Zeneca, Lilly and Novo-Nordisk. JDS received honoraria from MSD and Novo Nordisk and funding to attend meetings from Lilly, MSD, Novo Nordisk and Sanofi.

CONTRIBUTIONS

HS and PND conceived the hypothesis. HS, JDS, TS, YL performed laboratory analyses. , PND, HS, MKR, MG and YL designed the analysis and generated the results. HS, PND and SA wrote the first draft. MF, SK, JHH, and NY reviewed the first draft. All authors contributed to the final draft.

TRIAL REGISTRATION

This trial was registered in the ISRCTN Register. The trial registry number is ISRCTN58196433.

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TABLES AND FIGURES

Table 1. Data are mean (SD), median (IQR) or %, unless stated.

ACEI, angiotensin converting enzyme inhibitor; ARB, angiotensin receptor blocker; BMI, body mass index; DBP, diastolic blood pressure; eGFR, estimated glomerular filtration rate; MDRD, modification of diet in renal disease equation; SBP, systolic blood pressure.

Hypertension defined as BP>130/80 mmHg or anti-hypertensive therapy.

Comparisons of baseline clinical characteristics in low- and high-dose atorvastatin groups were not statistically significant.

Table 2. Data are mean (SD) or median (IQR).

AA, amyloid A; C, cholesterol; CETP, cholesterylester transfer protein; CRP, c-reactive protein; HDL, high density lipoprotein; LCAT, lecithin cholesterol acyltransferase; LDL, low density lipoprotein; Lp-PLA2, lipoprotein-associated phospholipase A2; PON, paraoxonase; TG, triglycerides.

Comparisons of baseline biomarkers in low- and high-dose atorvastatin groups were not statistically significant.

Table 3. Baseline data are mean (SD) or median (IQR). Change and difference data are mean (95% CI) values. The p-value considered statistically significant was decreased to <0.025 to allow for multiple testing. Data are adjusted for baseline level of biomarker, age, sex, MDRD eGFR (modification of diet in renal disease study estimated glomerular filtration rate), total cholesterol and HbA1c.

A negative value for adjusted mean difference indicates that patients receiving atorvastatin 80 mg daily had lower average values at 12 months than those receiving 10 mg daily. For example, LDL-C levels were 0.49 mmol/L lower ($p<0.001$) at 12 months in patients taking atorvastatin 80 mg daily compared to those taking 10 mg daily. A positive value for adjusted mean difference indicates that patients receiving atorvastatin 80 mg daily had higher average values during follow-up than those receiving 10 mg daily.

C, cholesterol; CEPT, cholesterylester transfer protein; CRP, c-reactive protein; HDL, high density lipoprotein; LCAT, Lecithin cholesterol acyltransferase; LDL, low density lipoprotein; Lp-PLA2, lipoprotein-associated phospholipase A2; PON, paraoxonase; Serum AA, serum amyloid A; TG, triglycerides; VLDL.

* $p < 0.025$ compared to baseline.

Table 4. Data are mean (SD) or median (IQR). Change data are mean (95% CI) values. The p-value considered statistically significant was decreased to <0.025 to allow for multiple testing. AA, amyloid A; C, cholesterol; CEPT, cholesteryl ester transfer protein; CRP, c-reactive protein; HDL, high density lipoprotein; LCAT, Lecithin cholesterol acyltransferase; LDL, low density lipoprotein; Lp-PLA2, lipoprotein-associated phospholipase A2; PON1, paraoxonase-1; TG, triglycerides.

Table 5. The percentage (95% CI's in parentheses) of patients achieving LDL cholesterol, non-HDL cholesterol and apolipoprotein B100 therapeutic goals on atorvastatin 10mg (n=59) and 80mg (n=60) daily after one year. Significantly more patients reached the therapeutic target for LDL and non-HDL cholesterol on 80mg daily (Chi squared test $P < 0.0001$ and <0.005 respectively). The proportion achieving the apolipoprotein B target of $<0.8\text{g/l}$ on the lower dose was not significantly greater than for LDL and for non-HDL cholesterol, but was similar when target of $<0.7\text{mg/l}$ was arbitrarily selected.

*Significantly different from % receiving atorvastatin 10mg daily (both $P < 0.05$). **Significantly different from % achieving LDL and non-HDL cholesterol targets on atorvastatin 10mg daily ($P < 0.05$).

Figure 1. Within-group percentage change from baseline for biomarkers in atorvastatin 10mg and 80mg groups. Statistical significance for changes between the two groups * $p < 0.025$, ** $p < 0.001$ (data are adjusted for baseline level of biomarker, age, sex, MDRD eGFR (modification of diet in renal disease study estimated glomerular filtration rate), total cholesterol, HbA1c, and use of washout period. The p-value considered statistically significant was decreased to <0.025 to allow for multiple testing. For more details on statistical significance of changes from baseline please see Table 3 and Table 4. apoAI, apolipoprotein AI; apoB, apolipoprotein B100; apoE, apolipoprotein E; CETP, cholesteryl ester transfer protein; high-sensitivity C-reactive protein; GlycaboB, glycated apolipoprotein B100; HDL-C, high density lipoprotein cholesterol; hsCRP, high-sensitivity C-reactive protein; LCAT, lecithin- cholesterol acyltransferase; LDL-C, low density lipoprotein cholesterol; Lp-PLA2, lipoprotein-associated phospholipase A2; Non-HDL-C, non-high density lipoprotein cholesterol; OxLDL, oxidized LDL; PON1, paraoxonase 1 activity; SAA, serum amyloid A; TC, total cholesterol; TG, triglycerides.

Figure1

Table 1. Baseline clinical characteristics at randomisation by treatment group

Baseline characteristic	Atorvastatin 10 mg	Atorvastatin 80 mg
<i>N</i>	59	60
Age (years)	64.5 (10.1)	63.5 (9.5)
Male (%)	81	85
Ethnicity (%)		
White European	80	95
South Asian	12	3
sBP (mm Hg)	134 (18)	129 (16)
dBp (mm Hg)	73 (10)	73 (9)
Hypertension (%)	98	96
HbA1c (mmol/mol)	61.3 (9.5)	59.5 (10.3)
HbA1c (%)	7.71 (1.21)	7.49 (1.29)
Diabetes therapy (%)		
Diet only	5	7
Oral hypoglycaemic agents	48	40
Insulin only	14	10
Insulin and oral hypoglycaemic agents	32	43
Diabetes duration (years)	12.6 (8.7)	11.1 (7.8)
BMI (kg/m ²)	32 (29-35)	34 (28-38)
Waist circumference (cms)	108 (13)	114 (18)
Smoker (%)	20	23
Retinopathy (%)	37	35
Neuropathy (%)	31	35
Coronary heart disease (%)	25	23
Cerebrovascular disease (%)	5	3
Peripheral arterial disease (%)	8	13
MDRD eGFR (mL/min/1.73 m ²)	61 (44-76)	72 (54-85)
Medication (%)		
ACEI	80	68
ARB	86	95
Calcium channel blocker (%)	64	57
B-blocker (%)	44	48
Thiazide (%)	56	57
Spironolactone (%)	15	7
Aspirin (%)	64	73

Table 1. Data are mean (SD), median (IQR) or %, unless stated.

ACEI, angiotensin converting enzyme inhibitor; ARB, angiotensin receptor blocker; BMI, body mass index; dBp, diastolic blood pressure; eGFR, estimated glomerular filtration rate; MDRD, modification of diet in renal disease equation; sBP, systolic blood pressure.

Hypertension defined as BP>130/80 mmHg or anti-hypertensive therapy.

Comparisons of baseline clinical characteristics in low- and high-dose atorvastatin groups were not statistically significant.

Table 2. Baseline biomarker data in high- and low-dose atorvastatin groups

Biomarker	Atorvastatin 10 mg daily N=59	Atorvastatin 80 mg daily N=60
Total cholesterol (mmol/l)	5.10 (1.02)	5.23 (1.11)
Triglycerides (mmol/l)	1.60 (0.95, 2.25)	1.80 (1.28, 2.33)
HDL-cholesterol (mmol/l)	1.20 (0.32)	1.20 (0.31)
LDL-cholesterol (mmol/l)	2.89 (0.83)	2.98 (0.80)
Non-HDL cholesterol (mmol/l)	3.90 (0.93)	4.02 (1.10)
Serum apo AI (g/l)	1.20 (0.25)	1.24 (0.20)
Serum apo B (g/l)	1.03 (0.22)	1.05 (0.22)
Small-dense LDL apo B (mg/dl)	18.3 (15.1, 21.5)	19.1 (14.49, 24.6)
Glycated apo B (mg/l)	5.25 (1.71)	5.11 (1.71)
Oxidised LDL (U/l)	25.5 (9.3)	28.3 (10.6)
Apo E (mg/l)	40.8 (24.6, 67.5)	45.8 (33.5, 66.6)
LCAT activity (nmol/ml/h)	41.8 (32.1, 60.9)	44.1 (29.3, 60.5)
CETP activity (nmol/ml/h)	19.6 (13.0, 30.0)	18.7 (12.2, 26.0)
Serum PON1 activity (nmol/ml/min)	116 (51,176)	94 (46,174)
Lp-PLA2 mass (ng/ml)	464 (365, 541)	473 (412, 534)
Adiponectin (mg/l)	1.8 (1.1, 2.9)	2.1 (1.25, 3.7)
High sensitivity CRP (mg/l)	2.33 (1.07, 3.72)	2.91 (1.57, 5.14)
Serum AA (mg/l)	33.5 (13.9, 68.5)	36.45 (16.4, 78.3)
Fasting glucose (mmol/l)	8.27 (2.48)	7.82 (2.86)
Cholesterol efflux (%)	6.19 (3.45)	5.84 (5.84)

Table 2. Data are mean (SD) or median (IQR).

AA, amyloid A; C, cholesterol; CETP, cholesterylester transfer protein; CRP, c-reactive protein; HDL, high density lipoprotein; LCAT, lecithin cholesterol acyltransferase; LDL, low density lipoprotein; Lp-PLA2, lipoprotein-associated phospholipase A2; PON1, paraoxonase1; TG, triglycerides.

Comparisons of baseline biomarkers in low- and high-dose atorvastatin groups were not statistically significant.

Table 3: Biomarker endpoints: 12-month data and multivariable-adjusted mean (95% CI) differences between high- and low-dose atorvastatin groups at 12 months follow-up.

Biomarker	12-month Atorvastatin 10 mg daily N=59	12-month Atorvastatin 80 mg daily N=60	Adjusted mean (95% CI) difference between high- and low-dose groups at 12 months	P-value for adjusted mean difference between- groups at 12 months
Total cholesterol (mmol/l)	4.12 (1.04)***	3.47 (0.89)***	-0.69 (-1.03 to -0.35)	<0.001
Triglycerides (mmol/l)	1.40 (1.0,1.8)***	1.13 (0.80,1.80)***	-0.31 (-0.57 to -0.05)	0.022
HDL-cholesterol (mmol/l)	1.26 (0.37)*	1.21 (0.30)	-0.05 (-0.19 to 0.03)	NS
LDL-cholesterol (mmol/l)	1.96 (0.63)***	1.50 (0.76)***	-0.49 (-0.73 to -0.25)	<0.001
Non-HDL cholesterol (mmol/l)	2.86 (0.89)***	2.26 (0.86)***	-0.66 (-0.98 to -0.34)	<0.001
Serum apo AI (g/l)	1.28 (0.29)	1.20 (0.21)	-0.10 (-0.18 to -0.02)	0.02
Serum apo B (g/l)	0.76 (0.20)***	0.65 (0.20)***	-0.12 (-0.18 to -0.06)	<0.001
Small-dense LDL apo B (mg/dl)	15.0 (12.3, 20.4)**	14.7 (12.6,18.1)***	-0.69 (-3.89 to 2.50)	NS
Glycated Apo B (mg/l)	3.96 (1.28)***	2.53 (1.21)***	-1.52 (-2.04 to -0.99)	<0.001
Oxidised LDL (U/l)	18.4 (8.5)***	17.2 (6.2)***	-3.5 (-6.5 to -0.5)	0.023
Apo E (mg/l)	37.2 (22.6,61.7)	31.9 (23.2, 49.4)***	-16.1 (-26.9 to -5.4)	0.004
LCAT activity (nmol/ml/h)	49.1 (40.9, 59.3)	50.2 (36.1, 64.6)	-0.1 (-6.8 to 6.6)	NS
CETP activity (nmol/ml/h)	18.1 (13.6, 24.0)	17.4 (11.4, 22.2)	-1.9 (-5.1 to 1.3)	NS
Serum PON1 activity (nmol/ml/min)	111 (52,176)	100 (46,161)	-2 (-12 to 8)	NS
Lp-PLA2 mass (ng/ml)	316 (237,405)***	264 (222,328)***	-55 (-84 to -26)	<0.001
Adiponectin (mg/l)	2.30 (1.55, 3.85)***	2.35 (1.55, 3.85)*	-0.17 (-0.60 to 0.35)	NS
High sensitivity CRP (mg/l)	2.30 (1.35, 3.95)	2.25 (1.20, 5.45)	-1.39 (-4.48 to 1.71)	NS
Serum AA (mg/l)	25.6 (10.5, 55.1)	20.7 (8.15, 64.05)*	-10.7 (-41.5 to 20.0)	NS
Fasting glucose (mmol/l)	7.59 (2.71)*	7.12 (2.70)	-0.04 (-0.93 to 0.85)	NS
HbA1c (mmol/mol)	60.0 (10.8)	60.3 (10.0)	0.8 (-2.1 to 3.7)	NS
HbA1c (%)	7.64 (1.37)	7.67 (1.27)	0.10 (-0.27 to 0.48)	NS
Cholesterol efflux (%)	6.47 (4.37)	8.54 (9.83)	1.20 (-2.47 to 4.87)	NS

Table 3. Baseline data are mean (SD) or median (IQR). Change and difference data are mean (95% CI) values. The p-value considered statistically significant was decreased to <0.025 to allow for multiple testing. Data are adjusted for baseline level of biomarker, age, sex, MDRD eGFR (modification of diet in renal disease study estimated glomerular filtration rate), total cholesterol and HbA1c.

A negative value for adjusted mean difference indicates that patients receiving atorvastatin 80 mg daily had lower average values at 12 months than those receiving 10 mg daily. For example, LDL-C levels were 0.49 mmol/L lower ($p<0.001$) at 12 months in patients taking atorvastatin 80 mg daily compared to those taking 10 mg daily. A positive value for adjusted mean difference indicates that patients receiving atorvastatin 80 mg daily had higher average values during follow-up than those receiving 10 mg daily.

C, cholesterol; CEPT, cholesterylester transfer protein; CRP, c-reactive protein; HDL, high density lipoprotein; LCAT, Lecithin cholesterol acyltransferase; LDL, low density lipoprotein; Lp-PLA2, lipoprotein-associated phospholipase A2; PON, paraoxonase; Serum AA, serum amyloid A; TG, triglycerides.

* $p<0.025$ compared to baseline.

Table 4. Unadjusted mean changes between 12 month and baseline for all patients (10 mg and 80 mg/day groups combined).

Biomarker	Baseline Combined Atorvastatin 10 mg and 80 mg daily N=119	12-month Combined Atorvastatin 10 mg and 80 mg daily N=119	Unadjusted mean (95% CI) changes between 12 month and baseline for all patients (10 mg and 80 mg/day groups combined)	P-value for unadjusted mean difference between- baseline and 12 month (10 mg and 80 mg/day groups combined)
Total cholesterol (mmol/l)	5.16(1.06)	3.80(1.02)	-1.42 (-1.63 to -1.20)	<0.001
Triglycerides (mmol/l)	1.70 (1.2, 2.4)	1.25(0.90,1.800)	-0.60 (-0.76 to -0.43)	<0.001
HDL-cholesterol (mmol/l)	1.2(0.313)	1.233(0.333)	0.024 (-0.018 to 0.065)	NS
LDL-cholesterol (mmol/l)	2.94(0.81)	1.74(0.73)	-1.22 (-1.37 to -1.08)	<0.001
Non-HDL cholesterol (mmol/l)	3.96 (1.02)	2.56 (0.92)	-1.44 (-1.64 to -1.23)	<0.001
Serum apo AI (g/l)	1.22(0.23)	1.24(0.25)	0.01 (-0.03 to 0.05)	NS
Serum apo B (g/l)	1.04(0.22)	0.71(0.21)	-0.34 (-0.37 to -0.30)	<0.001
Small-dense LDL apo B (mg/dl)	18.7 (14.7,23.5)	14.8 (12.3,19.5)	-3.18 (-5.00 to -1.35)	<0.001
Glycated Apo B (mg/l)	5.18(1.70)	3.26(1.43)	-1.90 (-2.40 to -1.40)	<0.001
Oxidised LDL (U/l)	26.8(10.0)	17.8(7.5)	-9.4 (-11.4 to -7.4)	<0.001
Apo E (mg/l)	41.4 (30.0, 67.0)	32.0 (22.6, 59.4)	-12.0 (-19.2 to -4.8)	0.003
LCAT activity (nmol/ml/h)	43.7 (31.6, 60.8)	49.8 (38.4, 62.1)	-0.24 (-5.59 to 5.11)	NS
CETP activity (nmol/ml/h)	18.8 (13.0 ,29.6)	17.9 (12.3, 22.8)	-3.5 (-6.2 to -0.9)	0.028
Serum PON1 activity (nmol/ml/min)	111 (49,176)	107 (50,172)	0 (-5 to 5)	NS
Lp-PLA2 mass (ng/ml)	469 (395, 536)	276 (230, 372)	-162 (-180 to -145)	<0.001
Adiponectin (mg/l)	1.90 (1.20, 3.30)	2.30 (1.55, 3.85)	0.41 (0.18 to 0.66)	<0.001
High sensitivity CRP (mg/l)	2.57 (1.11, 4.14)	2.3 (1.25, 4.70)	0.37 (-1.16 to 1.90)	NS
Serum AA (mg/l)	33.8 (15.1, 76.3)	22.1 (9.2, 61.2)	5.6 (-22.3 to 11.1)	0.004
Fasting glucose (mmol/l)	8.05(2.68)	7.36(2.70)	-0.73 (-1.24 to -0.22)	0.005
HbA1c (%)	7.68(1.30)	7.66(1.31)	0 (-0.18 to 0.19)	NS
HbA1c (mmol/mol)	60.3 (10.2)	60.2 (10.3)	0.03 (-1.41 to 1.47)	NS
Cholesterol efflux (%)	6.02(4.73)	7.47(7.53)	1.52 (-0.50 to 3.53)	NS

Table 4. Data are mean (SD) or median (IQR). Change data are mean (95% CI) values. The p-value considered statistically significant was decreased to <0.025 to allow for multiple testing.

AA, amyloid A; C, cholesterol; CETP, cholesterylester transfer protein; CRP, c-reactive protein; HDL, high density lipoprotein; LCAT, lecithin cholesterol acyltransferase; LDL, low density lipoprotein; Lp-PLA2, lipoprotein-associated phospholipase A2; PON1, paraoxonase-1; TG, triglycerides.

Table 5: Proportion of trial participants achieving lipid goals in low- and high-dose atorvastatin groups at 12 months

Target	Atorvastatin 10mg	Atorvastatin 80mg
LDL cholesterol <1.8mmol/l	29 (17, 41)%	68 (56, 80)%*
Non-HDL cholesterol <2.6mmol/l	40 (27, 53)%	72 (61, 83)%*
Apolipoprotein B100 <0.8g/l	70 (58, 82)% **	82 (72, 92)%
Apolipoprotein B100 <0.7g/l	36 (24, 48)%	68 (56, 80)%

Data are % (95% CI) *Significantly different from % receiving atorvastatin 10mg daily (both P<0.05). ** Significantly different from % achieving LDL and non-HDL cholesterol targets on atorvastatin 10mg daily (P<0.05).