

The effect of atorvastatin on pancreatic beta cell requirement in women with polycystic ovary syndrome

Running title: Atorvastatin on β cell requirement in PCOS

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Abstract

Background

There is an increased risk of developing T2DM in women with polycystic ovary syndrome (PCOS) and there is evidence that statins improve metabolic parameters in these patients. However, there is some data to show that statins increase the risk of incipient diabetes.

Material and Methods

We have previously shown that 12-weeks of atorvastatin improves insulin resistance when measured using HOMA-IR. This post hoc-analysis was designed to look at the effect of atorvastatin on pancreatic β cell function using HOMA- β in the same study. In this randomised, double blind placebo controlled study, 40 medication naïve patients with PCOS were randomized to either atorvastatin 20 mg daily or placebo for 3 months. A 3-month extension study for both groups of patients was undertaken with metformin 1500 mg daily after completing initial 3 months of atorvastatin or placebo.

Results

There was a significant reduction in HOMA- β (240 ± 3.2 vs. 177 ± 2.3 ; p -value < 0.01) after 12 weeks of atorvastatin treatment which was maintained by metformin in the subsequent 12 weeks. There were no changes in HOMA- β after the placebo or after subsequent metformin treatment.

There was no linear correlation between reduction in HOMA- β with improvement of free androgen index (FAI) ($r^2=0.02$; $p=0.72$), testosterone ($r^2=0.13$; $p=0.49$), SHBG ($r^2=0.22$; $p=0.48$), hsCRP ($r^2=0.19$; $p=0.64$), triglycerides ($r^2=0.09$; $p=0.12$), total cholesterol ($r^2=0.11$; $p=0.32$) or LDL-C ($r^2=0.19$; $p=0.38$).

Conclusion

Treatment with atorvastatin for 12 weeks in women with PCOS significantly reduced HOMA- β . This could be potentially due to fall in β cell requirement with improvement of insulin resistance rather than a reduction of β cell function.

Background

Statin (HMG-CoA reductase) treatment is effective in the primary and secondary prevention of cardiovascular disease (CVD) events ^{1, 2} and is generally safe and well tolerated ². In the West of Scotland Coronary Study (WOSCOPS) pravastatin treatment decreased the risk of type 2 diabetes (T2DM) by 30%³. Emerging evidence, however, suggests that treatment with other statins slightly increase the risk of T2DM ^{4 5}. In pooled data sets statin therapy was associated with a 9% increased risk of diabetes⁶ and this effect was age and dose dependent⁷. Population-based studies have also reported a 10–22% increased risk of diabetes with statins ⁸. However, there is also increasing evidence that reduction in insulin resistance especially with weight loss in patients with T2DM will result in normalisation of pancreatic β -cell requirement and even reversal of T2DM ⁹.

Statins have shown to improve certain features of polycystic ovary syndrome (PCOS) such as hyperandrogenemia ¹⁰⁻¹³. PCOS is one of the most common endocrine disorders that affects 6-20% of reproductive-aged women ¹⁴⁻¹⁶ and is at an increased risk of T2DM ^{17, 18}. Seventy five to 90% of PCOS patients demonstrate insulin resistance (IR) above and beyond that predicted by body mass, race, or age ^{19, 20} resulting in compensatory hyperinsulinaemia ²¹ and an increased risk for (T2DM) ²² and cardiovascular disease ²³. However, the effect of statins in improving insulin resistance is variable ^{10-13, 24}. Insulin resistance in most of these studies used HOMA-IR, which is a derivative use to measure insulin resistance from insulin and glucose. We have previously shown that 12 weeks of atorvastatin improves insulin resistance when measured using HOMA-IR ^{10, 11}. This post-hoc analysis was designed to look at the effect of atorvastatin in insulin secretion

using HOMA- β in the same study and its correlation with other hormonal and metabolic parameters.

Materials and Methods

A randomized double blind placebo controlled study was undertaken using atorvastatin 20mg daily¹¹. An extension arm followed this where immediately after stopping the study medication, 37 patients (19 patients from the atorvastatin group and 18 patients from the placebo group) who completed the study were given metformin 500mg three times daily for 3 months¹⁰. Clinical and biochemical assessments were performed at baseline, at the end of the 3-month period and at the end of 6 months after the extension phase.

The diagnosis of PCOS was based on all three diagnostic criteria of the Rotterdam consensus, namely clinical and biochemical evidence of hyperandrogenemia (Ferriman-Gallwey score >8; free androgen index >8 respectively), oligomenorrhoea or amenorrhoea and polycystic ovaries on transvaginal ultrasound²⁵. Subjects had no concurrent illness, were not on any medication for the preceding 6 months and were not planning to conceive. All patients gave written informed consent. The study was approved by the Hull and East Riding Local Research Ethics committee.

Study bloods and measurement were done after an overnight fast. Compliance was monitored by counting returned medication. Fasting venous blood was collected into serum gel and fluoride oxalate tubes. Samples were separated by centrifugation at 2000 g for 15 min at 4°C, and the aliquots stored at -20°C. Analytical methods have been described previously¹¹. The insulin resistance was calculated using the homeostasis

model assessment (HOMA) method ($\text{HOMA-IR} = (\text{insulin} \times \text{glucose})/22.5$). Pancreatic beta cell function (HOMA- β) was calculated using ($\text{HOMA-}\beta = (20 \times \text{insulin})/\text{glucose} - 3.5$). Data are reported as mean \pm SEM.

Statistical analysis

The sample size calculation for the original study was based on the known effects of atorvastatin on CRP in patients with impaired fasting glucose with the assumption that a similar effect occurs in those with PCOS^{11,26}. For 90% power, significance level of 5% and adjusting for a possible 20% dropout rate, 20 subjects were enrolled in each group.

Comparisons between both the groups from baseline were carried out using the paired t test for biochemical data and clinical observations. The Wilcoxon signed rank test was applied to biochemical data that violated the assumptions of normality when tested using the Kolmogorov-Smirnov test. Between-group comparison of percent changes was performed using independent samples t test. Intention to treat analysis was done. For all analysis, a two-tailed $P \leq 0.05$ was considered to indicate statistical significance. Statistical analysis was performed using SPSS for Windows NT, version 14.0 (SPSS Inc., Chicago, IL).

Results:

Thirty seven patients completed the study. Two patients from the placebo group and one patient from atorvastatin group dropped out of the study due to non-compliance.

The mean age group of patients was 27.7 ± 1.4 years (atorvastatin 26.6 ± 1.2 vs. placebo 28.8 ± 1.8). The BMI were comparable in both atorvastatin and placebo group (33.20 ± 1.4 vs. $33.92 \pm 1.4 \text{ kg/m}^2$).

There was a significant reduction in serum insulin levels and HOMA-IR in patients taking atorvastatin whilst there was significant increase in both these parameters in patients randomised to placebo. In the extension phase there were significant reduction in insulin and the HOMA-IR with metformin in the atorvastatin pre-treated group whereas, no significant changes in any of these parameters with metformin in the placebo pre-treatment group.

However, there was a significant reduction in HOMA- β ($240. \pm 3.2$ vs. 177 ± 2.3 ; p value <0.01) after 12 weeks of atorvastatin treatment compared to placebo, which was maintained by metformin in the subsequent 12 weeks (177 ± 2.3 vs. 185 ± 3.3 ; p value 0.42). There were no changes in HOMA- β after the placebo (Table 1).

There was no linear correlation between reduction in HOMA- β with improvement of FAI ($r^2=0.02$; p=0.72), testosterone ($r^2=0.13$; p=0.49), SHBG ($r^2=0.22$; p=0.48), hsCRP ($r^2=0.19$; p=0.64), triglycerides ($r^2=0.09$; p=0.12), total cholesterol ($r^2=0.11$; p=0.32) or LDL-C ($r^2=0.19$; p=0.38).

Discussion

In women with PCOS 12 weeks treatment with atorvastatin 20mg resulted in a significant reduction in pancreatic beta cell requirement as assessed by HOMA- β . This was independent of the improvement seen in the inflammatory markers, insulin resistance, lipids and hyperandrogenemia.

There was 26% reduction in pancreatic beta cell requirement as assessed by HOMA- β with atorvastatin treatment in this study compared to 20% reduction in insulin resistance as measured by HOMA-IR. It has been shown that in patients with T2DM, severe calorie restriction reverse T2DM with reduction of insulin resistance and normalisation of

pancreatic β -cell requirement⁹. After 12 weeks of atorvastatin in this study there was a significant improvement in insulin resistance as measured by HOMA-IR. This reduction in insulin resistance would have resulted in fall in the β -cell requirements. Another possibility is that the statin could have a direct deleterious effect on β -cells by multiple mechanisms. Statins have been shown to inhibit this glucose-induced calcium signaling-dependent insulin secretion²⁷. In addition, statins suppress the synthesis of ubiquinone (CoQ₁₀), an essential factor in the mitochondrial electron-transfer system, resulting in inhibition of insulin secretion due to reduced production of ATP²⁸. However, it is unlikely that the β -cell function fell more than expected by the reduction in insulin resistance since there was no significant hyperglycaemia after 12 weeks of atorvastatin therapy as measured by fasting glucose. This reduction in HOMA- β was maintained after subsequent metformin treatment. However, there were no significant changes in HOMA- β after placebo or subsequent metformin treatment.

This study used atorvastatin that is a lipid-soluble statin. There could be a differential effect of statins depending on lipid solubility rather than a class effect. Lipophilic statins such as simvastatin and atorvastatin are possibly absorbed by extra-hepatic cells; these statins can deregulate cholesterol metabolism, thus attenuating β -cell function²⁹. However, in the West of Scotland Coronary Study (WOSCOPS) treatment with pravastatin which is a water-soluble statin decreased the risk of diabetes by 30%³. There are currently no studies reported looking into the effect of non-lipophilic statins in women with PCOS.

There was no significant correlation with reduction of lipids, inflammatory markers, and hormonal parameters with reduction in HOMA- β . This shows that the effect of atorvastatin on HOMA- β is independent of its lipid lowering effect.

The limitation of the study includes the fact that this is a post-hoc analysis, however, all the biochemical analysis was done immediately after the study so that it should not affect the validity of analysis. For measurement of pancreatic beta cell function, hyperglycaemic insulin clamp studies or intravenous glucose tolerance test ideal, however in this study we calculated HOMA- β using fasting glucose and insulin measurements. HOMA- β correlates well with other methods of assessment of pancreatic beta cell function over a wide range of glucose tolerance status ($r=0.69$ vs. hyperglycemic clamp; $r=0.62-0.90$ for normal glucose tolerance; $r=0.73-0.88$ for impaired glucose tolerance; $r=0.69-0.90$ for diabetes)^{30,31}. However, more elaborate studies are needed to ascertain the effect of atorvastatin on beta cell function including insulin clamp studies.

In conclusion, treatment with atorvastatin for 12 weeks in women with PCOS significantly reduced HOMA- β . This could be potentially due to fall in β cell requirement with improvement of insulin resistance rather than suppression of β cell function, since there were no significant hyperglycaemia or hyperinsulinemia after atorvastatin therapy.

Declaration of interest: All authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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Author contribution statement (optional): TS, AMC, ESK and SLA conceived the study, done the analysis and drafted the manuscript.

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Table 1. Comparison of anthropometric and hormonal parameters at baseline, 12 weeks of atorvastatin or placebo followed by 12 weeks of metformin.

Parameter	Atorvastatin group						Placebo group					
	Baseline (V1)	12 weeks (V2) (Atorvastatin 20mg daily)	24 weeks (V3) (Metformin 1.5gm daily)	V1/V2 (pvalue)	%change V1-V2	V1/V3 (p value)	Baseline (V1)	12 weeks (V2) (Placebo)	24 weeks (V3) (Metformin 1.5gm daily)	V1/V2 (p value)	%change V1-V2	V1/V3 (p value)
Weight (kg)	91.29 ± 3.4	91.20 ± 3.4	90.3 ± 3.1	0.42	0.1 ± 0.1	0.08	93.12 ± 4.8	93.05 ± 4.7	92.6 ± 3.2	0.67	0.03 ± 0.02	0.12
Testosterone (nmol/L)	4.1 ± 0.2	2.9 ± 0.1	2.7 ± 0.1	<0.01	-24.6 ± 2.6	<0.01	4.4 ± 0.2	4.3 ± 0.2	4.2 ± 0.8	0.73	-0.1 ± 1.5	0.18
SHBG (nmol/L)	31.1 ± 1.0	35.3 ± 1.2	36.6 ± 1.7	<0.01	13.7 ± 2.4	<0.01	31.9 ± 0.9	32.9 ± 0.9	32.2 ± 0.8	0.14	3.6 ± 2.0	0.8
FAI	13.4 ± 0.6	8.7 ± 0.4	7.6 ± 0.9	<0.01	-32.7 ± 2.6	<0.01	13.9 ± 0.6	13.3 ± 0.5	13.1 ± 0.9	0.07	-- 3.1 ± 2.0	0.14
Glucose (mmol/L)	4.8 ± 0.1	4.9 ± 0.1	4.6 ± 0.1	0.52	1.0 ± 2.0	0.47	4.7 ± 0.5	4.8 ± 0.1	4.6 ± 0.4	0.32	2.0 ± 1.0	0.33
Insulin (µIU/mL)	15.6 ± 1.8	12.4 ± 1.7	10.2 ± 1.8	<0.01	-21.4 ± 4.0	<0.01	14.4 ± 2.0	17.6 ± 2.4	16.4 ± 1.8	0.04	34.2 ± 19.7	0.32
HOMA-IR	3.3 ± 0.4	2.7 ± 0.4	2.0 ± 0.8	<0.01	-19.8 ± 5.2	0.04	3.0 ± 0.4	3.8 ± 0.5	3.4 ± 0.3	0.04	37.2 ± 20.4	0.36
HOMA-β	240. ± 3.2	177 ± 2.3	185 ± 3.3	<0.01	-26.3 ± 1.1	0.07	240 ± 4.2	270 ± 8.2	298 ± 3.6	0.10	12.5 ± 1.0	0.08
TC (mmol/L)	4.6 ± 0.2	3.4 ± 0.2	3.9 ± 0.5	<0.01	-26.4 ± 3.0	0.82	4.5 ± 0.2	4.6 ± 0.2	4.5 ± 0.3	0.13	3.1 ± 1.1	0.82
LDL-C (mmol/L)	2.9 ± 0.2	1.8 ± 0.2	2.8 ± 0.4	<0.01	-36.6 ± 5.0	0.04	2.7 ± 0.2	2.7 ± 0.1	2.9 ± 0.3	0.77	3.1 ± 0.9	0.70
HDL-C (mmol/L)	1.07 ± 0.1	1.08 ± 0.1	1.07 ± 0.2	0.17	-4.0 ± 3.9	0.20	1.1 ± 0.08	1.1 ± 0.09	1.1 ± 0.1	0.47	3.0 ± 3.2	0.70
TG (mmol/L)	1.34 ± 0.08	1.08 ± 0.1	1.01 ± 0.1	<0.01	-20.9 ± 3.8	0.05	1.39 ± 0.24	1.69 ± 0.27	1.34 ± 0.1	0.19	36.8 ± 21.0	0.82

Atorvastatin group – Atorvastatin for 12 weeks followed by Metformin for 12 weeks.

Placebo group – Placebo for 12 weeks followed by Metformin for 12 weeks.

V1 – Baseline; V2 – 12 weeks from baseline on either atorvastatin or placebo; V3 - 24 weeks from baseline (12 weeks from visit 2 on Metformin 1.5g daily)

p - p value for percentage difference between both group using unpaired t test*

**pvalue < 0.01*

Data are presented as mean ± SEM. All serum results are obtained from fasting variables.

All variables were normally distributed

To convert values for testosterone to nanograms per deciliter, divide by 0.03467.

To convert values for SHBG to micrograms per deciliter, divide by 34.7.

To convert values for glucose to milligrams per deciliter, divide by 0.056.

To convert values for insulin to picomoles per liter, multiply by 6.

To convert values for cholesterol to milligrams per deciliter, divide by 0.0259.

To convert values for triglycerides to milligrams per deciliter, divide by 0.0113.

TC - Total cholesterol; LDL-C - LDL-cholesterol; HDL-C - HDL cholesterol; TG-Triglycerides; FAI – Free Androgen Index