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Atorvastatin Increases 25-Hydroxy Vitamin D Concentrations in Patients with Polycystic Ovary Syndrome

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BACKGROUND: It has been shown that many women with polycystic ovary syndrome (PCOS) are 25-hydroxyvitamin D (25OHD) insufficient. Both statin treatment and vitamin D supplementation have been shown to improve biochemical hyperandrogenemia, insulin resistance, and markers of inflammation in patients with PCOS, raising the possibility that some of the statin effects are mediated through vitamin D.

METHODS: We conducted this randomized, double-blind placebo controlled study to assess the effect of atorvastatin on serum 25OHD concentrations in patients with PCOS. Forty medication-naive patients with PCOS were randomized to either atorvastatin 20 mg daily or placebo for 3 months. After completing the initial 3 months of atorvastatin or placebo, both groups of patients participated in a 3-month extension study with metformin 1500 mg daily. We measured changes in 25OHD concentrations by use of tandem mass spectrometry.

RESULTS: Mean (SD) baseline 25OHD concentrations were comparable between the 2 groups [45.9 (2.4) vs 44.8 (1.8) nmol/L; $P = 0.7$]. There was a significant increase in 25OHD concentrations with atorvastatin [45.9 (2.4) vs 60.8 (3.5) nmol/L] compared with placebo [44.8 (1.8) vs 41.8 (3.2) nmol/L; $P = 0.02$]. Three-month treatment with metformin maintained the improvement of 25OHD with atorvastatin compared to baseline [45.9 (2.4) vs 61.8 (3.5), $P \leq 0.01$). There were no significant changes in 25OHD concentrations in the placebo group after 12 weeks of metformin.

CONCLUSIONS: Among patients with polycystic ovary syndrome, 12 weeks of atorvastatin led to a clinically significant rise in 25OHD concentrations. This may represent a beneficial pleiotropic effect of statins on 25OHD concentrations.

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Polycystic ovary syndrome (PCOS)[4] is a common disorder of women of reproductive age, affecting more than 10% of white women *(1)*, and is associated with increased prevalence of several cardiovascular risk factors *(2–8)*. Low serum concentrations of 25-hydroxyvitamin D (25OHD) are associated with higher cardiovascular risk, even after controlling for factors known to be associated with coronary artery disease *(9, 10)*. In women with PCOS, low 25OHD concentrations are associated with obesity and insulin resistance *(11)*. It has also been shown that many women with PCOS are 25OHD insufficient, and that 25OHD replacement therapy may have a beneficial effect on insulin resistance in obese women with PCOS *(12)*.

Statins have been shown to significantly reduce cardiovascular morbidity and mortality in hypercholesterolemic patients in both primary and secondary prevention *(13)*. It has been suggested that the striking benefit achieved with statin treatment in patients with a wide range of cholesterol concentrations cannot be attributed to their cholesterol-lowering effect alone, but rather to additional pleiotropic effects. One of these pleiotropic effects may be mediated in part by an effect on 25OHD metabolism *(14)*, and there is some evidence that statins can improve 25OHD concentrations in patients with familial hypercholesterolemia and ischemic heart disease *(15–18)*.

We have already shown that atorvastatin improves biochemical hyperandrogenemia, insulin resistance, and markers of inflammation in patients with polycys-

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[4] Nonstandard abbreviations: PCOS, polycystic ovary syndrome; 25OHD, 25-hydroxyvitamin D; LC-MS/MS, liquid chromatography–tandem mass spectrometry; MRM, multiple reaction monitoring; hsCRP, high-sensitivity C-reactive protein; HOMA-IR, homeostatic model assessment–insulin resistance.

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tic ovary syndrome *(19)*. This study was done to investigate whether any of these changes were mediated by the effect of atorvastatin on 25OHD concentrations in patients with PCOS.

Materials and Methods

The diagnosis of PCOS was based on all 3 diagnostic criteria of the Rotterdam consensus, namely clinical and biochemical evidence of hyperandrogenemia (Ferriman–Gallwey score >8 and free androgen index >8, respectively), oligomenorrhea or amenorrhea, and polycystic ovaries on transvaginal ultrasound *(20)*. Study participants had no concurrent illness, were not on any medication for the preceding 9 months (except study medications), and were not planning to conceive. None of the patients had successful pregnancy or miscarriage for at least 5 years before study entry. Participants were advised not to change their lifestyle, including physical activity or dietary habits, during the study period. Nonclassic 21-hydroxylase deficiency, hyperprolactinemia, Cushing disease, and androgen-secreting tumors were excluded by appropriate tests. The study participants were recruited consistently at times spread throughout the year to negate the effect of seasonal changes in 25OHD concentrations. None of the patients was on any medication or vitamin D supplements. Basal dietary intake was not formally assessed at baseline, but none of the patients were vegans. All the patients were white and came from the same geographic area, having similar sunlight exposure. All patients gave informed consent. The study was approved by the South Humber Research Ethics committee.

We randomized 40 medication-naive patients with PCOS and biochemical hyperandrogenemia to atorvastatin 20 mg daily or placebo for 3 months *(19)*. We undertook an extension study for both groups of patients with metformin 1500 mg daily after completing the initial 3 months of atorvastatin or placebo *(21)*. Thirty-seven patients (atorvastatin 19; placebo 18) completed 6 months of this study.

Blood samples were taken after an overnight fast, and serum was stored frozen at −80°C pending analysis. We monitored compliance by counting returned medication.

We measured 25OHD concentrations by use of isotope-dilution liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS) *(22)*. The selectivity/specificity of the method for vitamin D is based on 2 multiple reaction monitoring (MRM) transitions, specific retention time (HPLC selectivity), and sample preparation and mass spectrometer tuning optimized for vitamin D. Nonetheless, to help ensure that atorvastatin was not interfering with the vitamin D method, we analyzed a 100-mg/L solution of atorvastatin (at least 1000 times the concentrations encountered in plasma for patients taking standard doses of atorvastatin) with no corresponding mass fragments being seen. Indeed, chromatography of the same solution showed a complete absence of peaks discernable from baseline noise.

There are circumstances in which the specificity of the LC-MS/MS may be compromised, especially for separation of stereoisomers. LC-MS/MS assays have been found to measure 3-epi-$25OHD_3$, which is a naturally occurring vitamin D isomer found in infants, and good chromatographic resolution is required for its measurement by LC-MS/MS. Atorvastatin is an unrelated compound, however, and its known metabolites differ in mass and structure from $25OHD_3$; therefore, in terms of both chromatographic and mass resolution, the application of LC-MS/MS in this situation seems entirely rational.

STATISTICAL ANALYSIS

We used paired *t*-test to compare changes from baseline for the biochemical data and clinical observations within groups. We applied the Wilcoxon signed-rank test to biochemical data that violated the assumptions of normality when tested using the Kolmogorov–Smirnov test. We evaluated the effect of treatment by first computing the percentage change from baseline in all variables studied and then the percentage change for each variable in both groups, thus negating the differences in the baseline values of the 2 groups. We performed between-group comparison of percent changes using independent samples *t*-test. For all analyses, a 2-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.). Data are reported as mean (SE).

Results

The mean (SD) age of the patients was 27.7 (1.4) years [atorvastatin 26.6 (1.2) vs placebo 28.8 (1.8)]. Body mass index measurements were comparable in the atorvastatin and placebo groups [33.20 (1.4) vs 33.92 (1.4) kg/m^2].

The 25OHD method measured both $25OHD_2$ and $25OHD_3$. In all patients, $25OHD_2$ was below the limit of quantification (<5.0 nmol/L), and therefore total 25OHD was a measure of $25OHD_3$.

Baseline 25OHD concentrations were comparable between the groups [45.9 (2.4) vs 44.8 (1.8) nmol/L; $P = 0.7$]. There was a significant increase of 25OHD concentrations with atorvastatin compared to placebo (Table 1). Three-month treatment with metformin maintained the improvement of 25OHD with atorva-

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Table 1. Comparison of mean (SD) 25OHD, calcium, phosphate, alkaline phosphatase, and hsCRP at baseline and after atorvastatin or placebo followed by metformin.[a]

	Baseline (V1)	After 12 weeks (V2)	After 24 weeks (V3)	Change, % (V1 to V2)	*P*, V1 vs V2	*P*, V2 vs V3	*P*, V1 vs V3
Atorvastatin pretreatment (n = 19)							
25OHD, nmol/L	45.9 (2.4)	60.8 (3.5)	61.7 (2.8)	47.0 (0.9)[b]	<0.01	0.66	<0.01
Calcium, mmol/L	2.38 (0.2)	2.42 (0.3)	2.42 (0.8)	1.6 (0.8)	0.32	0.47	0.38
Phosphate, mmol/L	0.99 (0.08)	1.2 (0.02)	0.97 (0.09)	20.2 (0.2)	0.48	0.52	0.64
Alkaline phosphatase, IU/L	40.2 (1.2	43.2 (0.9)	42.9 (1.1)	7.4 (0.4)	0.39	0.48	0.54
hsCRP, mg/L	4.9 (1.4)	3.4 (1.1)	2.7 (0.8)	−15 (0.1)[c]	0.04	0.04	0.02
Placebo pretreatment (n = 18)							
25OHD, nmol/L	44.8 (1.8)	41.8 (3.2)	42.1 (2.2)	−1.0 (0.3)	0.72	0.44	0.32
Calcium, mmol/L	2.40 (0.7)	2.39 (0.9)	2.44 (0.2)	−10.0 (0.1)	0.44	0.53	0.69
Phosphate, mmol/L	0.97 (0.05)	0.98 (0.09)	0.98 (0.11)	1.2 (0.2)	0.12	0.32	0.49
Alkaline phosphatase, IU/L	39.8 (0.9)	42.7 (0.6)	43.1 (1.2)	7 (0.9)	0.19	0.62	0.33
hsCRP, mg/L	5.8 (1.4)	5.7 (1.6)	5.2 (1.8)	−5.0 (0.8)	0.90	0.66	0.58

[a] Atorvastatin or placebo for 12 weeks followed by metformin for 12 weeks. *P* values by paired *t*-test.
[b] <0.01 (each *P* value given in footnotes b and c is for the difference in percentage changes between the atorvastatin and placebo treatment groups by use of unpaired *t*-test).
[c] <0.05 (see footnote b).

statin compared to baseline [45.9 (2.4) baseline vs 61.8 (3.5) nmol/L for atorvastatin treatment; $P < 0.01$]. There were no significant changes in 25OHD concentrations in the placebo group after 12 weeks of metformin.

There was a significant correlation between the increase in 25OHD with a reduction in high-sensitivity C-reactive protein (hsCRP) ($r = 0.80$, $P = 0.02$). No correlation was observed between increase in serum 25OHD concentrations with an improvement in total cholesterol ($r = 0.34$, $P = 0.3$), triglycerides ($r = 0.27$, $P = 0.12$), homeostatic model assessment–insulin resistance (HOMA-IR) ($r = 0.33$, $P = 0.2$), or free androgen index ($r = 0.42$, $P = 0.1$). No changes in alkaline phosphatase, adjusted calcium, or phosphate were observed between groups (Table 1).

Discussion

In patients with PCOS, 12-week treatment with atorvastatin at a dose of 20 mg daily resulted in a significant increase in serum 25OHD concentrations that was independent of the lipid-lowering effect of atorvastatin. There was a significant correlation between the increases in 25OHD concentrations and the reduction of hsCRP.

Low concentrations of 25OHD are associated with a higher risk of myocardial infarction in a graded manner, even after controlling for factors known to be associated with coronary artery disease *(10)*. Several studies have shown that increased exposure to sunlight protects against coronary heart disease through production of 25OHD *(23, 24)*. Furthermore, the only dietary change that consistently protects against CHD is an increase in consumption of oily fish and fish oil, which contain large amounts of 25OHD *(25, 26)*. The present study has shown an increase in 25OHD concentrations directly in response to atorvastatin indicative of the postulated statin pleiotropic effect, suggesting that the clinical benefit of statins may also be mediated through the protective effect of increased 25OHD concentrations in addition to their cholesterol-lowering effects.

Owing to the importance of calcium in both oocyte activation and maturation, it has been hypothesized that abnormalities in calcium and 25OHD homeostasis may play a role in the pathogenesis of PCOS *(27)*. 25OHD receptors are expressed in the ovary and testis, suggesting that 25OHD is active in these organs *(28)*. It has been shown that calcium and 25OHD supplementation improves menstrual irregularities in patients with PCOS *(27)*. In a pilot study, it appeared that the combined use of calcium and 25OHD plus metformin was more effective for correcting menstrual disorders and maturation of follicles than either drug alone in patients with PCOS *(29)*. In the current study, metformin maintained the improvement of 25OHD concentrations in atorvastatin-pretreated patients. However, it is difficult to determine if menstrual regu-

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larity was affected in this study, as it was too short for a reliable effect.

The increase in 25OHD concentrations had a significant correlation with reduction of hsCRP, which is an inflammatory marker. Mounting evidence suggests that, in addition to its well-described roles in skin, bone, and muscle physiology *(30)*, the hormone 25OHD acts as an inhibitor of the inflammatory response through several pathways *(31)*. Decreased 25OHD concentrations have been associated with an increased risk of developing autoimmune diseases, such as multiple sclerosis, rheumatoid arthritis, and type 1 diabetes *(32–35)*. 25OHD administration has been shown to prevent the initiation and attenuate the severity of immune-mediated diseases, including type 1 diabetes *(36, 37)* and animal models for multiple sclerosis *(38)*. In addition, it has been shown that 25OHD decreased rheumatoid arthritis disease activity *(39)*. Furthermore, an inverse relation has been shown between 25OHD concentrations and CRP, a marker of inflammation, in both healthy individuals and patients with rheumatoid arthritis *(40)*. In this study, there was a significant increase in 25OHD concentrations with atorvastatin, which correlated with improvement of hsCRP.

It is interesting to note that cholesterol and 25OHD have the same precursor, namely 7-dehydrocholesterol. Hence, it might be expected that there would be a reduction of 25OHD synthesis with statins, although the opposite was shown in this study. A potential mechanism to explain this paradox could be the antiinflammatory action of statins. Statins have been shown to have a beneficial effect in reducing infective or inflammatory episodes *(41)*, in a pattern similar to that of 25OHD *(14)*. It has been observed that blood 25OHD concentrations are low in patients with tuberculosis and remain low throughout treatment but increase afterward spontaneously *(42)*. Hypothetically, statins may suppress the inflammatory processes that would consume vitamin D, leading to its serum increase.

No matter what the mechanism is, this study has shown that 12 weeks of atorvastatin treatment increased 25OHD concentrations in patients with polycystic ovary syndrome. Their effect on 25OHD concentrations may to an extent explain the beneficial pleiotropic effects of statins.

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