Use of statins is associated with lower serum total and non-sex hormone-binding globulin-bound testosterone levels in male participants of the Rotterdam Study

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Abstract

Objective: Statins, or HMG-CoA reductase inhibitors, decrease cholesterol production. Because cholesterol is a precursor of the testosterone biosynthesis pathway, there is some concern that statins might lower serum testosterone levels. The objective of the present study was to investigate the association between the use of statins and serum testosterone levels in men.

Design: Cross-sectional study within the prospective population-based Rotterdam Study.

Subjects and methods: We included 4166 men with available data on total testosterone, non-sex hormone-binding globulin (SHBG)-bound testosterone, and medication use. Multivariable linear regression analysis was used to compare the differences in serum testosterone levels (nmol/l) between current, past, and never statin users. We considered dose and duration of use. Analyses were adjusted for age, BMI, cardiovascular disease, diabetes mellitus, hypertension, and estradiol levels.

Results: We identified 577 current (mean age 64.1 years), 148 past (mean age 64.6 years), and 3441 never (mean age 64.6 years) statin users. Adjusted for all covariables, current statin use of 1–6 months or >6 months was significantly associated with lower total testosterone levels as compared to non-users (β = 1.24, 95% CI 2.17, 0.31, and β = 1.14, 95% CI 2.07, 0.20 respectively). Current use of 1–6 months was also associated with significantly lower non-SHBG-bound testosterone levels (β = 0.42, 95% CI 0.82, 0.02). There was a trend toward lower testosterone levels at higher statin doses both for total (P_trend 2.9×10^-5) and non-SHBG-bound (P_trend 2.0×10^-6) testosterone. No association between past statin use and testosterone levels was found.

Conclusion: We showed that current use of statins was associated with significantly lower serum total and non-SHBG-bound testosterone levels. The clinical relevance of this association should be further investigated.

Introduction

Statins, or HMG-CoA reductase inhibitors, are widely used cholesterol-lowering drugs that are effective in the primary and secondary prevention of cardiovascular disease (CVD) (1, 2, 3). They competitively inhibit HMG-CoA reductase, the rate-limiting enzyme in the cholesterol biosynthesis pathway, mainly in the liver. By inhibiting
this cholesterol biosynthesis, the number of LDL receptors in the hepatic membrane is increased. This leads to increased serum uptake of LDL cholesterol and thus to a decrease in serum cholesterol concentration (4).

Testosterone is the main circulating androgenic hormone in men, and it has important effects on libido, bone mass, fat distribution, muscle mass, strength, and the production of blood cells and sperm (5, 6). It is synthesized in the testes, and this process requires a continuous supply of cholesterol, which can be derived from plasma that mostly originates from the liver or from de novo production within the gland (7). In contrast, women have in general eight to nine times lower levels of serum total testosterone; the hormone also plays a role in sexual function and libido in women (8). Circulating testosterone in men is 40–65% bound to sex hormone-binding globulin (SHBG), which regulates the serum concentration of testosterone and its transport to target tissues. SHBG has a high binding affinity for testosterone, and the serum concentrations of total testosterone and SHBG are strongly correlated. In contrast, the non-SHBG-bound fraction of testosterone, which is considered to be bioactive, is barely associated with serum SHBG concentrations (9, 10, 11). This indicates that non-SHBG-bound testosterone, rather than total testosterone, plays an important role in maintaining equilibrium in the negative feedback of the hypothalamo–pituitary–testicular axis and in other androgenic effects.

Because statins decrease cholesterol biosynthesis, and because cholesterol is the precursor of testosterone, there is some concern about whether statins might impair testosterone production. Statins decrease serum availability of the substrate cholesterol; in vitro studies have shown that statins decrease cholesterol production in testicular Leydig cells (12) or inhibit enzymes within the testosterone biosynthesis pathway (e.g., 17β-hydroxysteroid dehydrogenase) (13). A lower testosterone level resulting from statins may be undesired in men who already have a low testosterone level, because it may lead to symptoms such as a decrease in mood, libido, muscle strength, or bone mineral density. The 2013 American guidelines for CVD prevention lowered the threshold for treatment with statins and widened the target population (14). Furthermore, the prevalence of diseases such as type 2 diabetes mellitus (T2DM) and CVD is increasing (15, 16). Therefore, the already substantial use of statins in clinical practice may further increase in the future, and this might be accompanied by an increase in the non-beneficial effects of statins (17). Consequently, it is important to elucidate whether statins decrease serum testosterone levels and, more specifically, non-SHBG-bound testosterone, which is a potential undesired effect of their use.

In the present large population-based cohort study, our objective was to investigate whether the use of statins was associated with decreased serum levels of total and non-SHBG-bound testosterone in males aged 45 years or older.

Subjects and methods
Setting and study population

The present research was conducted within the Rotterdam Study, a prospective population-based cohort study that aims to examine the frequency and determinants of diseases in middle-aged and elderly people. The rationale and design of the Rotterdam Study have been described previously (18, 19).

In short, at start, all 10 275 persons aged ≥55 years in the Ommoord district of Rotterdam, The Netherlands, were invited to participate. Of them, 7983 (78%) were enrolled between 1990 and 1993 (RS-I). At baseline, participants underwent extensive clinical examinations at the research center. Since then, participants have been followed during up to four follow-up rounds (1993–1995, 1997–1999, 2002–2004, and 2009–2012). In 2000, an extended cohort was enrolled (RS-II), in which 3011 inhabitants aged ≥55 years entered the study, and they have been continuously followed since then. Furthermore, in 2006, a third cohort (RS-III) was formed, and it included 3932 inhabitants aged ≥45–54 years at enrollment.

The Rotterdam Study has been approved by the medical ethics committee according to the Wet Bevolkingsonderzoek: ERGO (Population Study Act: Rotterdam Study), which was executed by the Ministry of Health, Welfare and Sports of the Netherlands. All participants gave informed consent to participate in the study and to provide information from their treating physicians and pharmacies.

All three cohorts of the Rotterdam Study were considered in the present research, which included a study population aged 45 years and older. The study population consisted of all male participants in the Rotterdam Study for whom a serum total testosterone measurement was available, for whom a serum non-SHBG-bound testosterone concentration could be calculated, and for whom medication-dispensing data were available (n = 4255). Seeing as most of the women in the Rotterdam Study are postmenopausal and have very low testosterone levels, we expected that any potential
effect of statins would probably only be demonstrated in males and/or would not be clinically relevant in females. Therefore, we restricted our study population to only male participants.

**Outcome assessment**

Our outcomes of interest were the serum levels of total testosterone and non-SHBG-bound testosterone. Serum hormone data were assessed from laboratory measurements during the third visit of the first cohort (RS-I-3, 1997–1999), the first visit of the second cohort (RS-II-1, 2000–2001), and the first visit of the third cohort (RS-III-1, 2006–2008). Serum levels of non-SHBG-bound testosterone were used as a measure of bioactive testosterone.

**Exposure assessment**

The exposure of interest was the use of statin therapy. Medication-dispensing data were obtained from all seven fully computerized linked pharmacies in the Ommoord district. Information on all of the prescriptions filled from January 1, 1991, until February 1, 2012, was available and included information on the product name of the drug, the Anatomical Therapeutical Chemical code (ATC code), the amount dispensed, the prescribed dose regimen, and the date of dispensing ([http://www.whocc.no/atc_ddd_index](http://www.whocc.no/atc_ddd_index); accessed October 6, 2014). For every dispensing of a statin, the duration of use (prescription episode) was calculated by dividing the number of dispensed tablets by the prescribed daily number. Repeated prescriptions, which were filled within 7 days after ending the previous filled prescription, were considered continuous use.

On the date of the testosterone measurement, every participant was classified into one of the following mutually exclusive categories: ‘current use’ if the measurement occurred within a prescription episode; ‘past use’ if the participant had previously stopped using statins; or ‘never use’ if the participant had not used statins during the study period. Current and past statin users were further stratified into five categories according to the duration of their exposure to statins: current use ≤ 1 month; current use > 1 to ≤ 6 months and current use > 6 months; past use > 6 months; and past use ≤ 6 months since the end of the last prescription episode. The 6-month cutoff was applied based on the median duration of current and past use in the population. To facilitate direct dose comparisons between drugs from the same therapeutic drug group, the daily dose of statin therapy was expressed in ‘defined daily doses’ (DDD; [http://www.whocc.no/atc_ddd_index](http://www.whocc.no/atc_ddd_index); accessed October 6, 2014).

**Analytical determinations**

All steroids and SHBG were estimated in the same serum sample obtained from blood taken in the morning in the fasting state. Testosterone, DHEA, and DHEAS were measured simultaneously with an LC-MS/MS method using the CHS MSMS Steroids kit (Perkin Elmer, Turku, Finland). The steroids kit uses a combined solvent extraction and protein precipitation method with acetonitrile that contains the deuterated internal standards $^2$H$_5$-testosterone, $^2$H$_6$-DHEA, and $^2$H$_6$-DHEAS. The internal standard underwent processing identical to the analytes. The chromatographic separation was performed on a Waters (Milford, MA, USA) Acquity UPLC HSS T3 1.8 μm column (diameter 1 mm, length 10 cm) and in-line filter 0.2 μm frit with an acetonitrile/Methanol gradient. A Waters XEVO-TQ-S system equipped with an ESI source operating in the electrospray positive mode was used for quantitation. The lower limits of quantitation for testosterone, DHEA, and DHEAS were 0.07, 2.2, and 24.7 nmol/l respectively. Non-SHBG-bound testosterone was calculated according to the method of Sodergard et al. (20) using previously described equations (9) and assuming a fixed albumin level of 40 g/l. SHBG, estradiol (E$_2$), and insulin were measured by a Cobas 8000 Modular Analyzer (Roche Diagnostics GmbH). Fasting total cholesterol was measured by a Cobas c702 system (Roche Diagnostics GmbH).

**Covariables**

Variables were considered to be either potential confounders or effect modifiers that influence the association between the use of statins and serum testosterone levels based on their clinical relevance or their confounding effect in the analysis (a variable that changed the estimate > 10% was considered to be a confounder) (21, 22, 23). Our selected confounders were: age, BMI, a history of CVD, T2DM, hypertension, and serum E$_2$ levels. Testosterone is known to decrease with increasing age and BMI. CVD, hypertension, and T2DM are conditions that are associated with lower testosterone levels. E$_2$ changed the estimate > 10% and was also correlated with testosterone and SHBG. Furthermore, analyses were adjusted for the prescribed DDD of statin therapy.

In addition, we investigated whether additional adjustment for insulin, total cholesterol, DHEA, or DHEAS influenced the association between SHBG and testosterone, and we investigated whether these variables were effect modifiers or intermediates. Insulin influences SHBG levels, which are strongly correlated with total
testosterone; total cholesterol and DHEA are part of the pathway from cholesterol to testosterone; and DHEAS is a metabolite of DHEA.

BMI was calculated as weight (in kg) divided by height squared (in m²). A history of CVD was defined as the occurrence of a myocardial infarction, percutaneous transluminal coronary angioplasty, coronary artery bypass grafting, heart failure, carotid deobstruction, cerebrovascular accident, or transient ischemic attack before the date of testosterone measurement (24, 25, 26). T2DM was defined as a current prescription for an oral glucose-lowering drug or insulin (ATC code A10). Hypertension was defined as blood pressure higher than 140/90 mmHg or a current prescription for an antihypertensive agent (ATC code C02, C03, C07, C08 or C09; http://www.whocc.no/atc_ddd_index; accessed October 6, 2014).

A sensitivity analysis was performed in participants without CVD in order to investigate whether the associations are constant in men without CVD. Another sensitivity analysis was performed in which the most-recent past users (within 14 days) from the ≤6 months past use group were reallocated into the current use category. This was done because we believed that patients who were recently exposed to statins might still be affected by the drug (i.e., carry-over effect). In another sensitivity analysis, we adjusted the analyses on statins and total testosterone for serum SHBG levels to investigate whether SHBG might play a role in the association between statins and total testosterone.

Statistical analyses were performed using SPSS Software version 21.0 (SPSS, Inc.). All P values are two-sided and were considered statistically significant when P<0.05.

Results

In total, 4255 male participants with a serum total testosterone measurement, a calculated serum non-SHBG-bound testosterone measurement, and medication-dispensing data were available. We analyzed complete case sets, so 89 (2%) of the 4255 participants were excluded because of incomplete data on the covariates. Of all the 4166 eligible male participants in the study population, 577 (14%) were current statin users and 148 (3.5%) were past statin users. Characteristics of the current, past, and never users are shown in Table 1. All of the hormone levels were non-normally distributed in the population and were therefore log transformed in the analyses. Compared to never users, current and past users had significantly higher BMIs and higher prevalences of CVD, T2DM, and hypertension. Furthermore, current statin users had significantly higher serum insulin and E2 levels and significantly lower levels of SHBG, DHEA, DHEAS, and cholesterol. Both current and past statin users had significantly lower mean total (current users 13% ↓, past users 9% ↓) and non-SHBG-bound (current users 8% ↓, past users 7% ↓) testosterone levels as compared to never users. The majority of current statin users used simvastatin (n=345, 59.8%), followed by atorvastatin (n=112, 19.4%), pravastatin (n=68, 11.8%), fluvastatin (n=34, 5.9%), and rosuvastatin (n=18, 3.1%).

In the multivariable linear regression analysis, after adjustment for all of the covariates, current use of statins was associated with statistically significantly lower total testosterone levels as compared to never use, with a β of −1.18 nmol/l (95% CI −1.96, −0.40, P=0.003). Past statin use was not significantly associated with lower total
testosterone levels as compared to never use ($\beta = 0.79$, 95% CI $1.70$, 0.12, $P=0.089$) (Table 2). When duration of use was considered, >1 to $\leq$ 6 months current use and >6 months current use were associated with statistically significantly lower total testosterone levels as compared to never use ($\beta = 1.24$, 95% CI $2.17$, $-0.31$, $P=0.009$; >6 months current use: $\beta = 1.14$, 95% CI $2.07$, $-0.20$, $P=0.017$). For the past use categories and $\leq$ 1 month current use, no significant association with serum total testosterone levels was found (Table 2).

In the multivariable analyses on non-SHBG-bound testosterone levels, current statin use was associated with lower non-SHBG-bound testosterone levels as compared to never use ($\beta = 0.35$, 95% CI $-0.68$, $-0.01$, $P=0.042$). Past statin use was not significantly associated with lower non-SHBG-bound testosterone levels as compared to never use ($\beta = 0.26$, 95% CI $-0.65$, 0.13, $P=0.191$) (Table 2). When duration of use was considered, >1 to $\leq$ 6 months current use was associated with statistically significantly lower non-SHBG-bound testosterone levels ($\beta = 0.42$, 95% CI $-0.82$, $-0.02$, $P=0.039$). For the other past and current use categories, no significant association was found (Table 2).

We investigated whether other hormones and total cholesterol were intermediates in the association between statins and testosterone levels. Additional adjustment for insulin and DHEAS did not change the results significantly. Additional adjustment for total cholesterol and DHEA showed the largest effects on the associations and reduced the magnitude of the effect. Additional adjustment for total cholesterol showed a $\beta$ of $-0.97$ (95% CI $-1.75$, $-0.18$, $P=0.015$) for total testosterone and a $\beta$ of $-0.25$ (95% CI $-0.58$, 0.90, $P=0.151$) for non-SHBG-bound testosterone for current use, instead of the original $\beta$s of $-1.18$ and $-0.35$ respectively. Additional adjustment for DHEA showed similar results to those for total cholesterol adjustment (data not shown).

Moreover, we stratified statin DDD in tertiles to investigate whether a higher statin dose was associated with a stronger testosterone-lowering effect. As shown in Fig. 1, there was a trend toward a stronger testosterone-lowering effect at a higher statin dose, both for total testosterone ($P_{\text{trend}} 2.9 \times 10^{-5}$) and non-SHBG-bound testosterone ($P_{\text{trend}} 2.0 \times 10^{-5}$). Current statin use in the mid- and high-dosage tertiles was associated with $\beta$s of $-1.17$ nmol/l (95% CI $-2.00$, $-0.35$, $P=0.005$) and $-1.89$ nmol/l (95% CI $-2.75$, $-1.03$, $P=1.7 \times 10^{-5}$) of lower serum total testosterone respectively as compared to never users. For non-SHBG-bound testosterone, current statin use in the mid- and high-dosage tertiles was associated with $\beta$s of $-0.44$ nmol/l (95% CI $-0.79$, $-0.09$, $P=0.015$).
Table 2  Multivariable linear regression on the association between the use of statin therapy and serum total and non-SHBG-bound testosterone levels. Bold values indicate a statistically significant association.

<table>
<thead>
<tr>
<th></th>
<th>(\beta) (s.e.m.)(^a)</th>
<th>95% CI</th>
<th>P value</th>
<th>n</th>
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</thead>
<tbody>
<tr>
<td><strong>Total testosterone</strong></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Current, past, and never use(^b)</td>
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<tr>
<td>Never use</td>
<td>(ref)</td>
<td>–</td>
<td>–</td>
<td>3441</td>
</tr>
<tr>
<td>Current use</td>
<td>–1.18 (0.40)</td>
<td>–1.96, –0.40</td>
<td>0.003</td>
<td>577</td>
</tr>
<tr>
<td>Past use</td>
<td>–0.79 (0.47)</td>
<td>–1.70, 0.12</td>
<td>0.089</td>
<td>148</td>
</tr>
<tr>
<td>Current and past use(^c)</td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Never use</td>
<td>(ref)</td>
<td>–</td>
<td>–</td>
<td>3441</td>
</tr>
<tr>
<td>(\leq 1) month current use</td>
<td>–1.13 (0.71)</td>
<td>–2.52, 0.26</td>
<td>0.111</td>
<td>78</td>
</tr>
<tr>
<td>&gt; 1–6 months current use</td>
<td>–1.24 (0.47)</td>
<td>–2.17, –0.31</td>
<td>0.009</td>
<td>288</td>
</tr>
<tr>
<td>&gt; 6 months current use</td>
<td>–1.14 (0.48)</td>
<td>–2.07, –0.20</td>
<td>0.017</td>
<td>226</td>
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<tr>
<td>(\leq 6) months past use</td>
<td>–1.17 (0.62)</td>
<td>–2.39, 0.05</td>
<td>0.061</td>
<td>81</td>
</tr>
<tr>
<td>&gt; 6 months past use</td>
<td>–0.35 (0.67)</td>
<td>–1.67, 0.97</td>
<td>0.600</td>
<td>67</td>
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<tr>
<td><strong>Non-SHBG-bound testosterone</strong></td>
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<tr>
<td>Current, past, and never use(^b)</td>
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<tr>
<td>Never use</td>
<td>(ref)</td>
<td>–</td>
<td>–</td>
<td>3441</td>
</tr>
<tr>
<td>Current use</td>
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<td>–0.68, –0.01</td>
<td>0.042</td>
<td>577</td>
</tr>
<tr>
<td>Past use</td>
<td>–0.26 (0.20)</td>
<td>–0.65, 0.13</td>
<td>0.191</td>
<td>148</td>
</tr>
<tr>
<td>Current and past use(^c)</td>
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<td>Never use</td>
<td>(ref)</td>
<td>–</td>
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<td>3441</td>
</tr>
<tr>
<td>(\leq 1) month current use</td>
<td>–0.29 (0.30)</td>
<td>–0.89, 0.31</td>
<td>0.341</td>
<td>78</td>
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<tr>
<td>&gt; 1–6 months current use</td>
<td>–0.42 (0.20)</td>
<td>–0.82, –0.02</td>
<td>0.039</td>
<td>288</td>
</tr>
<tr>
<td>&gt; 6 months current use</td>
<td>–0.29 (0.21)</td>
<td>–0.70, 0.11</td>
<td>0.153</td>
<td>226</td>
</tr>
<tr>
<td>(\leq 6) months past use</td>
<td>–0.34 (0.27)</td>
<td>–0.87, 0.18</td>
<td>0.200</td>
<td>81</td>
</tr>
<tr>
<td>&gt; 6 months past use</td>
<td>–0.17 (0.29)</td>
<td>–0.73, 0.40</td>
<td>0.563</td>
<td>67</td>
</tr>
</tbody>
</table>

SHBG, sex hormone-binding globulin.

\(^a\)Analyses are adjusted for age, BMI, history of cardiovascular disease, diabetes mellitus, hypertension, estradiol level, and statin dose.

\(^b\)Current and past use of statin therapy compared to never use as the reference category.

\(^c\)Current and past use categories: the 1-month current use cutoff was applied to investigate whether statins already exert an effect after 1 month of therapy; the 6-month current and past use cutoff was applied based on the median duration of current and past use in the population.

and \(−0.85\) nmol/l (95% CI \(−1.21, −0.48, P=6.0\times10^{−6}\)) respectively as compared to never users.

In a sensitivity analysis, we excluded all participants with a history of CVD (8% of the population). Current users again showed significantly lower total testosterone levels (\(\beta = −1.12,\) s.e.m. 0.43, \(P=0.048\)). The effect estimate for current users on lower non-SHBG-bound testosterone levels was similar to the original analysis, although it was nonsignificant (\(\beta = −0.29,\) s.e.m. 0.27, \(P=0.125\)). For past statin use, again no association was found.

In an additional sensitivity analysis, we reallocated 23 participants from the \(\leq 6\) months past use group to the current use group. These patients were the most recent past users (they had stopped statins \(\leq 14\) days before testosterone assessment). Results showed no significant association of past use with lower total or non-SHBG-bound testosterone levels. Current users again showed a stronger and more significant association with total testosterone levels (\(\beta = −1.31, 95\%\) CI \(−2.06, −0.56, P=0.001\)) and non-SHBG-bound testosterone levels (\(\beta = −0.42, 95\%\) CI \(−0.74, −0.10, P=0.011\)) than that of never users (Table 3). When past users within 30 days (40 participants) were reallocated to current use, the results were similar to the past users within 14 days reallocation (data not shown).

In an additional sensitivity analysis, we also adjusted the analyses on statins and total testosterone for serum SHBG levels. The association between current statin use and lower serum total testosterone levels was attenuated but remained statistically significant (original analysis: \(\beta = −0.65, 95\%\) CI \(−1.27, −0.04, P=0.038\); sensitivity analysis with reallocated recent past users: \(\beta = −0.73, 95\%\) CI \(−1.32, −0.14, P=0.015\)).

**Discussion**

In the present cross-sectional population-based study, we showed that current use of statins was associated with statistically significantly lower levels of serum total and non-SHBG-bound testosterone in males. We demonstrated that the magnitude of the decrease in testosterone was directly proportional to the dosage of statin therapy. Considering the duration of statin use, the association between current statin use and lower testosterone levels
was present after at least 1 month of current use. For past use, no association was found with testosterone levels.

As far as we know, there have been five cross-sectional studies in the literature that studied the association between statins and testosterone levels. Three of those studies were in line with the present findings on total testosterone levels and showed that statins were associated with lower total testosterone levels (27, 28, 29), whereas two showed no association at all (30, 31). The studies that showed lower total testosterone levels demonstrated a difference of 1.5, 1.6, and 3.0 nmol/l respectively, whereas the mean total testosterone levels in the present study were 2.2 nmol/l lower in current users as compared to never users. Three of those five studies also investigated free testosterone; two did not find an association (28, 30), but one showed significantly lower free and bioavailable testosterone levels (29). Bioavailable testosterone levels were 1.0 nmol lower in statin users than those in non-users (29), whereas in the present study, mean non-SHBG-bound testosterone levels were 0.7 nmol/l lower in current users than they were in never users. The disadvantages of the previous studies as compared to the present study are that one did not adjust for potential confounders (28), another studied only 25 statin users (31), only one study provided statin dosage (29), and none of these studies analyzed the duration of therapy. Moreover, one of the studies was conducted only in patients who were consulting for erectile dysfunction (29), whereas in another of the studies, only T2DM patients were selected (28).

Similarly, placebo-controlled randomized trials have studied total testosterone levels before and after statin therapy. A recent meta-analysis that included five such trials concluded that current statin therapy (4 weeks to 3 months) induced a decrease in total testosterone of $-0.66$ nmol/l in men (32).

Theoretically, there are several mechanisms by which statins could inhibit testosterone production. The main one is by inhibiting HMG-CoA reductase in the testis (12). By operating in the testis, statins consequently suppress de novo cholesterol production, which impairs the substrate source for the testosterone biosynthesis pathway. The second mechanism is by decreasing serum cholesterol concentrations and therefore cholesterol uptake by the testis, which could again limit cholesterol availability and its impact on testosterone production. A third mechanism is by directly inhibiting other enzymes in the testosterone pathway.

### Table 3  Sensitivity analysis: multivariable linear regression on the association between the use of statin therapy and serum total testosterone levels with recent past users reallocated. Bold values indicate a statistically significant association.

<table>
<thead>
<tr>
<th>Total testosterone</th>
<th>$\beta$ (s.e.m.)$^a$</th>
<th>95% CI</th>
<th>P value</th>
<th>n</th>
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</thead>
<tbody>
<tr>
<td>Current, past, and never use$^b$</td>
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<tr>
<td>Never use</td>
<td>Ref</td>
<td>–</td>
<td>–</td>
<td>3441</td>
</tr>
<tr>
<td>Current use</td>
<td>$-1.31$ (0.38)</td>
<td>$-2.06$, $-0.56$</td>
<td>0.001</td>
<td>600</td>
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<tr>
<td>Past use</td>
<td>$-0.49$ (0.50)</td>
<td>$-1.47$, $0.49$</td>
<td>0.331</td>
<td>125</td>
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<tr>
<td>Non-SHBG-bound testosterone</td>
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<tr>
<td>Current, past, and never use$^b$</td>
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<td>Never use</td>
<td>Ref</td>
<td>–</td>
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<td>3441</td>
</tr>
<tr>
<td>Current use</td>
<td>$-0.42$ (0.16)</td>
<td>$-0.74$, $-0.10$</td>
<td>0.011</td>
<td>600</td>
</tr>
<tr>
<td>Past use</td>
<td>$-0.12$ (0.21)</td>
<td>$-0.54$, $0.30$</td>
<td>0.569</td>
<td>125</td>
</tr>
</tbody>
</table>

SHBG, sex hormone-binding globulin.

$^a$Analyses are adjusted for age, BMI, history of cardiovascular disease, diabetes mellitus, hypertension, estradiol level, and statin dose.

$^b$Current and past use of statin therapy compared to never use as the reference category.
biosynthesis pathway (13). An alternative theory for the decrease in testosterone levels is that statin users have increased insulin levels because they often have metabolic syndrome, and statins themselves may increase insulin levels (33). Insulin suppresses SHBG production in the liver (34, 35), which results in consequently lower total testosterone levels, because free testosterone levels are kept constant in response to the decrease in SHBG levels (9). This may be supported by the finding that the association between statins and total testosterone is attenuated by additional adjustment for SHBG in sensitivity analyses. However, no studies have described an effect of statin use on SHBG levels, and sensitivity analyses in which we additionally adjusted for DHEA, DHEAS, insulin, or cholesterol support the idea that the association between statins and testosterone is explained by statins lowering cholesterol. Additional adjustment for total cholesterol or DHEA attenuated the association between statins and lower testosterone levels, whereas DHEAS and insulin showed no significant effect. Both cholesterol and DHEA are substrates for testosterone formation, and attenuation of the association through additional adjustment supports the hypothesis that statins lower testosterone by reducing cholesterol. Also, DHEA is a reflection of adrenal steroid production, and suppression of DHEA may suggest an effect on adrenal steroidogenesis (36).

The suggestion that statins are testosterone-lowering drugs has been criticized (37), and several arguments were used to do so. First, a decrease of −0.66 nmol/l, as shown in the recent meta-analysis (32), is a small mean decrease in total testosterone level. Second, in the literature, there has been a poor correlation between the use of statins and symptoms such as decreased libido or muscle strength, which calls into question the clinical relevance of this decrease. However, there is variability in the response to statins, and some patients might be more vulnerable to statins and will have a stronger decrease (38). A modest average decrease in a population might hide a substantial decrease in a handful of individuals, and in those who already have low testosterone, it might be clinically meaningful. Moreover, even modest effects on a population-based scale could gain more relevance now that statin therapy is increasingly being used. For instance, applying the adapted American guidelines on male participants in the Rotterdam Study would entail prescribing statins to nearly all elderly men (i.e., 96.4%) (39). This corroborates the idea that adverse reactions to statins (e.g., a testosterone-lowering effect) deserve attention. Moreover, when the decrease in testosterone levels is clinically relevant, statin users can become eligible for testosterone replacement therapy (TRT). A possible association between TRT and an increase in cardiovascular events has been discussed in the literature. However, evidence for this association is controversial, and most studies showed no increased cardiovascular risk related to TRT (40, 41, 42, 43), whereas only one study demonstrated a possible risk (44). Nevertheless, the US Food and Drug Administration recently warned about the possible increased risk of heart attack and stroke in the treatment of low testosterone levels that result from aging, and it cautioned that TRT is approved only for men who have low testosterone levels caused by certain medical conditions (45).

The present study is cross-sectional because the regular assessment of serum testosterone levels is very unusual in a population-based setting. Nevertheless, we were able to study the duration of use in order to establish a temporal relationship. Current use for >1 to ≤6 months and for >6 months showed significantly lower total testosterone levels. The first month of therapy was investigated separately to gain insight into how testosterone levels behave during the beginning of statin use. During the first month, the testosterone-lowering effect was nonsignificant and less strong than it was for the other current use categories, which suggests that more time is needed to induce a complete effect on testosterone levels. After >1 to ≤6 months of statin use, non-SHBG-bound testosterone levels decreased in parallel with total testosterone. However, for the >6 months statin users, non-SHBG-bound testosterone levels also showed a negative β that was, however, not significant. As described in the introduction to the present study, non-SHBG-bound testosterone drives the negative feedback mechanism of the hypothalamo–pituitary–gonadal axis to keep its concentration constant. It is possible that in long-term statin users, the set point has adapted to the effect of statins on testosterone lowering and become a compensatory effect to prevent the levels of bioactive non-SHBG-bound testosterone from falling. This might explain why we could not demonstrate an effect of long-term statin use on the levels of bioactive non-SHBG-bound testosterone.

The magnitude of the association between current statin use and lower serum total and non-SHBG-bound testosterone was stronger at higher statin doses. This is in line with what is expected, and it strengthens our finding. Some misclassification of exposure may have occurred in very recent past users who may actually be current users that took a drug holiday or were subject to a carry-over effect after stopping the drug. In sensitivity analyses, the association was strengthened for current users, which
supports the possibility of some misclassification of exposure.

The strengths and potential limitations of the present study should be considered. The Rotterdam Study is a large, prospective population–based cohort study with extensive data collection. For instance, we were able to consider statin dose and were the first study to consider the effect of the duration of statin use. Furthermore, we accounted for potential confounding variables (e.g., T2DM, hypertension, and CVD). Compared to other cross-sectional studies, we managed not only to overcome potential flaws but also to further extend and strengthen the analysis. We adjusted for confounding variables by indicating which variables potentially play a role, because diseases such as CVD and T2DM have been independently associated with lower testosterone levels (46).

A strength of the present study is that we measured total testosterone, DHEA, and DHEAS by LC–MS/MS. Although we measured E2 by electrochemiluminescence immunoassay, the method used has been standardized via ID–GC/MS. In the Rotterdam Study, no direct measurement of free testosterone by equilibrium dialysis was performed. Instead, we calculated non-SHBG-bound testosterone using the formula of Sodergard et al. (20). Results from both types of estimations of free testosterone yielded highly correlated results (47). Another limitation is the lack of data on gonadotropin levels in the Rotterdam Study. Ideally, the study should be performed longitudinally with several testosterone assessments over time. However, this is very unusual in a population-based setting. Unfortunately, the number of current statin users was too low to investigate the association in users of the different types of statins separately. However, we expected a class effect of statins on testosterone lowering. The present study supports the hypothesis that statins lower testosterone by lowering cholesterol and thus suggests that all statins will show this effect. Analyses of the association between current statin use and testosterone levels showed a similar direction of the effect (negative β) for all statins separately. However, the association only remained significant for total testosterone using the formula of Sodergard (20). Results from both types of estimations of free testosterone yielded highly correlated results (47).

In conclusion, the present study showed that current use of statin therapy is associated with significantly lower serum total and non-SHBG-bound testosterone levels after adjustment for important confounders. Given the large number of statin-treated males and the important biological role of testosterone, the clinical relevance of this association should be further investigated.

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

Funding
The Rotterdam Study is supported by the Erasmus Medical Center and Erasmus University Rotterdam; the Netherlands Organization for Scientific Research (NWO); the Netherlands Organization for Health Research and Development (ZonMW); the Research Institute for Diseases in the Elderly (RIDE); the Netherlands Genomics Initiative (NGI); the Ministry of Education, Culture and Science; the Ministry of Health, Welfare and Sport; the European Commission (DG XII); and the Municipality of Rotterdam.

Author contribution statement
All authors contributed substantially to the manuscript and have met the criteria for authorship.

Acknowledgements
The contributions of the study participants, general practitioners, and pharmacists of the Ommoord district to the Rotterdam Study are gratefully acknowledged.

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Received 3 December 2014
Revised version received 8 April 2015
Accepted 5 May 2015