The efficacy of statins in preventing glucocorticoid-related osteonecrosis in animal models

A META-ANALYSIS

Objectives
The primary purpose of this meta-analysis was to determine whether statin usage could reduce the risk of glucocorticoid-related osteonecrosis in animal models.

Methods
A systematic literature search up to May 2015 was carried out using the PubMed, Ovid, EBM reviews, ISI Web of Science, EBSCO, CBM, CNKI databases with the term and boolean operators: statins and osteonecrosis in all fields. Risk ratio (RR), as the risk estimate of specific outcome, was calculated along with 95% confidence intervals (CI). The methodological quality of individual studies was assessed using a quantitative tool based on the updated Stroke Therapy Academic Industry Roundtable (STAIR) recommendations.

Results
A total of 11 eligible studies were included according to predetermined criteria. The pooled data demonstrated that animals with statin usage, either alone or combined with other treatments, were at a decreased risk of developing glucocorticoid-related osteonecrosis (RR = 2.06, 95% confidence interval (CI) 1.71 to 2.50). Moreover, subgroup analysis revealed that compared with statins alone, statins combined with other treatments significantly decreased the risk of osteonecrosis (RR = 1.23, 95% CI 1.02 to 1.47). However, we could find no significant risk difference for different gender, or for different time points.

Conclusions
The present study suggests that statins combined with other treatments are efficient in preventing the development of glucocorticoid-related osteonecrosis in animals. These results might shed light on clinical practice when glucocorticoids are prescribed, and could be further investigated in high-quality clinical trials.

Cite this article: Bone Joint Res 2016;5:393–402.

Keywords: Statins; Glucocorticoid; Osteonecrosis; Prevention; Animal model

Article focus
- Our study aimed to determine whether statin usage reduces the risk of glucocorticoid (GC)-related osteonecrosis (ON) in animal models.

Key messages
- Our results suggest that statin usage combined with other treatments could reduce the risk of developing GC-related ON in animal models.

Strengths and limitations
- This is the first meta-analysis to review the efficacy of statins in preventing GC-induced ON in animal models.
- No distinction was made for animal models and the type or dose of statins and GCs.

Introduction
Osteonecrosis (ON) is a common, progressive and devastating disease with an insidious onset. ON presents without specific clinical symptoms and signs and most commonly affects young adults in the third and fourth decade of their life. Management options for ON vary from joint salvaging procedures to joint replacement, and are based on the stages described by the Association of Research Circulation Osseous (ARCO) classification. Conservative treatment,
represented by core decompression, may be efficient in
the early stages and in small lesions of osteonecrosis
(stages I, II). However, concerns have been raised due to
the potential for core decompression to weaken the can-
cellous bone within and adjacent to the necrotic zones.4
With respect to joint replacement surgeries, approxi-
mately 10 000 to 20 000 new cases of ON are diagnosed
in the United States every year, and it is estimated that 5%
to 12% of total hip arthroplasties are performed every
year in order to treat this disease.1,2

Glucocorticoid (GC) usage is the leading cause of non-
traumatic ON of the femoral head.5 ON develops in 9% to
40% of patients who receive high-dose or long-term ster-
oid therapy.6 Despite the strong association of GC with
ON, the underlying mechanisms of ON have been
unclear. Several hypotheses have been introduced that
explain the mechanism of GC-induced ON. The proposed
pathogenesis includes lipid metabolism disturbance,
apoptosis, increased oxidative stress and disturbances of
the coagulation-fibrinolysis system due to steroid hor-
mones.1-3,7,8 In particular, intraosseous hypertension,
intravenous fat embolisms, and compression of vessels
by progressive accumulation of marrow fat store, are
commonly accepted theories.9 Based on these findings,
an increasing number of studies have been initiated to
explore the effects of lipid-lowering agents on preventing
ON.9,20

Statins (3-hydroxymethyl-3-glutaryl-CoA (HMG-CoA)
reductase inhibitors), widely used for the treatment of
hyperlipidemia as well as for preventing coronary artery
diseases,21,22 have been likewise an attractive candidate
for prevention of GC-induced ON. Beneficial effects do
not only result from lowering cholesterol level, but also
from pleiotropic effects including improvement of
endothelial dysfunction, antioxidant effects, reduction of
platelet activity and decreased bone cell apoptosis.21-23
These various effects of statins may play an important role in the prevention of GC-induced ON. Previous research studies\textsuperscript{14-16} have shown serum lipid levels were significantly lower in the statin group than in the control group, which received steroids only.

Interestingly, Iwakiri et al\textsuperscript{15} have shown that increased CYP3A activity owing to statins is a possible mechanism for the protective effect, given the fact that extrinsic glucocorticoids are made inactivate predominantly by hepatic CYP3A. Histological examination\textsuperscript{14-16} revealed that rabbits treated with steroids and statins maintained more physiological bone marrow fat cell size and fraction of marrow filled by fat. In vitro and in vivo studies\textsuperscript{9,18} demonstrated that statin acts on bone marrow mesenchymal stem cells and modulate their differentiation by enhancing osteogenesis through increasing expression of \textit{Cbfa1/Runx2} improving activity of the osteocalcin promoter, and inhibiting the adipogenesis through decreasing expression of the adipocyte-specific genes \textit{PPAR\gamma2} (fat cell transcription factor) and \textit{422aP} (fat-specific). Statins

**Table IV.** Characteristics of statin intervention of included studies

| Study ID | Types      | Statins administration | Dose (mg/kg.d.) | Duration (wks) | Route of delivery | Treatment point before steroid |
|----------|------------|------------------------|-----------------|----------------|-------------------|-------------------------------|
| Li et al 2014\textsuperscript{23} | atorvastatin | 2.5                   | 8               | Food admixture  | 0 wk             |
| Xie et al 2013\textsuperscript{26} | lovastatin  | 25                    | 14              | Gavage         | 2 wks            |
| Nozaki et al 2012\textsuperscript{13} | pravastatin | 15                    | 4               | Drinking water | 2 wks            |
| Li et al 2011\textsuperscript{27}  | pravastatin | 2.5                   | 12              | Food admixture | 0 wk             |
| Kang et al 2010\textsuperscript{14} | lovastatin  | 5                     | 14              | Food admixture | 2 wks            |
| Zeng et al 2009\textsuperscript{26} | simvastatin | 20 mg, daily          | NR              | PO             | 0 wk             |
| Iwakiri et al 2008\textsuperscript{15} | pravastatin/simvastatin | 2/5                  | 6/6             | PO/IV          | 3 wks/3 wks       |
| Pengde et al 2008\textsuperscript{16} | lovastatin  | 5                     | 14              | Food admixture | 2 wks            |
| Nishida et al 2008\textsuperscript{17} | pravastatin | 0.7                  | 4               | IV             | 2 wks            |
| Kang et al 2007\textsuperscript{29}  | lovastatin  | 300                   | 14              | Food admixture | 2 wks            |
| Cui et al 1997\textsuperscript{18}   | lovastatin  | 20 mg, daily          | NR              | PO             | 0 wk             |

PO, peros; IV, intravenous; NR, not reported
are also associated with an elevated bone morphogenetic protein-2 gene expression, alkaline phosphatase activity, matrix mineralisation, and enhanced osteogenesis by the bone cells in vitro. Although several animal and limited clinical studies indicated that statins may have a protective role against ON in a recent large-scale cohort study revealed that ON-free survival was similar in renal transplantation patients with and without statin exposure.

It is therefore not clear if statins could effectively prevent GC-related ON. Therefore, the primary purpose of this meta-analysis is to determine whether statin usage reduces the risk of GC-related ON in animal models.

**Materials and Methods**

**Search strategy.** An electronic search was conducted online to identify relevant studies up to May 2015, using the PubMed, Ovid MEDLINE(R) (1946 to present with daily update), all EBM reviews, ISI Web of Science, Academic Search Premier and MEDLINE in EBSCO, China Biological Medicine Database, and China National Knowledge Infrastructure databases with the following terms and boolean operators: statins and osteonecrosis in all fields. In addition, bibliographies of retrieved articles were searched by hand for further pertinent studies. Furthermore, we contacted

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**Table V.** The methodological quality of individual study

| Study ID          | (1) | (2) | (3) | (4) | (5) | (6) | (7) | Score |
|-------------------|-----|-----|-----|-----|-----|-----|-----|-------|
| Li et al 2014    | *   | *   | *   |     |     |     |     | 3     |
| Xie et al 2013   | *   |     |     |     |     |     |     | 3     |
| Nozaki et al 2012| *   |     |     |     |     |     |     | 5     |
| Li et al 2011    | *   |     |     |     |     |     |     | 2     |
| Kang et al 2010  | *   |     |     |     |     |     |     | 3     |
| Zeng et al 2009  | *   |     |     |     |     |     |     | 2     |
| Iwakiri et al 2008 | * |     |     |     |     |     |     | 4     |
| Pengde et al 2008 | * |     |     |     |     |     |     | 3     |
| Nishida et al 2008 | * |     |     |     |     |     |     | 3     |
| Kang et al 2007  | *   |     |     |     |     |     |     | 2     |
| Cui et al 1997   | *   |     |     |     |     |     |     | 2     |

*One score
Studies fulfilling the criteria of (1) sample size calculation; (2) inclusion and exclusion criteria; (3) randomisation; (4) allocation concealment; (5) reporting of animals excluded from analysis; (6) blinded assessment of ON; (7) reporting potential conflicts of interest and study funding

**Table VI.** Evaluation of osteonecrosis of included studies

| Study ID          | Diagnosis of osteonecrosis |
|-------------------|---------------------------|
|                   | Unilateral or bilateral   | Sample from | Methods               |
| Li et al 2014    | Bilateral                 | Femoral head | Radiological examination |
| Xie et al 2013   | NR                        | Femoral head | Histological examination |
| Nozaki et al 2012| Bilateral                 | Proximal femur | Histological examination |
| Li et al 2011    | Unilateral                | Femoral head | Histological examination |
| Kang et al 2010  | Bilateral                 | Femoral heads | Radiological examination |
| Zeng et al 2009  | Bilateral                 | Proximal femur | Histological examination |
| Iwakiri et al 2008 | Bilateral                | Femur and humerus* | Histological examination |
| Pengde et al 2008 | Bilateral                | Femur and humerus* | Histological examination |
| Nishida et al 2008 | Bilateral               | Femur and humerus* | Histological examination |
| Kang et al 2007  | Bilateral                 | Proximal femur | Histological examination |
| Cui et al 1997   | Bilateral                 | Femoral head | Histological examination |

*The whole area of the proximal one third and distal condyles of both the femur and the humerus
NR, not reported

**Table VII.** Outcomes of each included study

| Study ID          | Animals treated with statin and steroid | Animals treated with steroidalone |
|-------------------|-----------------------------------------|----------------------------------|
|                   | Osteonecrosis | Total | Osteonecrosis | Total |
| Li et al 2014    | 4            | 15    | 7            | 15    |
| Xie et al 2013   | 19           | 60    | 17           | 30    |
| Nozaki et al 2012| 11           | 15    | 23           | 23    |
| Li et al 2011    | 5            | 16    | 10           | 16    |
| Kang et al 2010  | 13           | 52    | 16           | 24    |
| Zeng et al 2009  | 1            | 20    | 3            | 19    |
| Iwakiri et al 2008 | 9          | 30    | 25           | 30    |
| Pengde et al 2008 | 9            | 25    | 18           | 26    |
| Nishida et al 2008 | 13         | 35    | 21           | 30    |
| Kang et al 2007  | 8            | 32    | 11           | 16    |
| Cui et al 1997   | 0            | 10    | 14           | 25    |
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| Study or Subgroup | Statin | Events | Total | Control | Events | Total | Weight | Risk Ratio | Subtotal (95% Cl) | Total (95% Cl) |
|-------------------|--------|--------|-------|---------|--------|-------|--------|------------|-----------------|----------------|
| Nozaki 2012       | 4      | 15     | 0     | 23      | 0.5%   | 13.50 (0.78 to 233.96) |        |
| Iwakiri 2008      | 21     | 30     | 5     | 30      | 5.7%   | 4.20 (1.82 to 9.67)    |        |
| Kang 2007         | 24     | 32     | 5     | 16      | 7.7%   | 2.40 (1.13 to 5.10)    |        |
| Kang 2010         | 39     | 52     | 8     | 24      | 12.6%  | 2.25 (1.25 to 4.05)    |        |
| Cui 1997          | 10     | 10     | 11    | 25      | 7.9%   | 2.16 (1.38 to 3.39)    |        |
| Nishida 2008      | 22     | 35     | 9     | 30      | 11.1%  | 2.10 (1.15 to 3.33)    |        |
| Kang 2008         | 16     | 25     | 8     | 26      | 9.0%   | 2.08 (1.09 to 3.97)    |        |
| Li 2011           | 11     | 16     | 6     | 16      | 6.9%   | 1.83 (0.90 to 3.74)    |        |
| Xie 2013          | 41     | 60     | 13    | 30      | 19.9%  | 1.58 (1.01 to 2.46)    |        |
| Zeng 2009         | 19     | 20     | 6     | 9       | 9.5%   | 1.43 (0.89 to 2.29)    |        |
| Li 2014           | 11     | 15     | 8     | 15      | 9.2%   | 1.38 (0.78 to 2.41)    |        |

**Fig. 2**

Forest plot showing the overall risk estimate of osteonecrosis between groups with or without statins (M-H, Mantel–Haenszel; CI, confidence interval; df, degrees of freedom). Risk ratio (RR) on the left axis indicates statin usage decreases the risk of osteonecrosis compared with the control group, whereas RR greater than 1 indicates animals with statin usage are at increased risk of osteonecrosis. The 95% CI reveals that the result is statistically significant when “1” is not included in the interval.

Total (95% CI) 310 244 100.0% 2.06 (1.71 to 2.50)

**Fig. 3**

Forest plot showing subgroup analysis based on study quality score (M-H, Mantel–Haenszel; CI, confidence interval; df, degrees of freedom). Risk ratio (RR) on the left axis indicates statin usage decreases the risk of osteonecrosis compared with the control group, whereas RR greater than 1 indicates animals with statin usage are at increased risk of osteonecrosis. The 95% CI reveals that the result is statistically significant when “1” is not included in the interval, and vice versa.

| Study or Subgroup | Statin | Events | Total | Control | Events | Total | Weight | Risk Ratio | Subtotal (95% Cl) | Total events |
|-------------------|--------|--------|-------|---------|--------|-------|--------|------------|-------------------|--------------|
| Nozaki 2012       | 4      | 15     | 0     | 23      | 0.5%   | 13.50 (0.78 to 233.96) | 154 51 |
| Iwakiri 2008      | 21     | 30     | 5     | 30      | 5.7%   | 4.20 (1.82 to 9.67)    |        |
| Kang 2007         | 24     | 32     | 5     | 16      | 7.7%   | 2.40 (1.13 to 5.10)    |        |
| Kang 2010         | 39     | 52     | 8     | 24      | 12.6%  | 2.25 (1.25 to 4.05)    |        |
| Cui 1997          | 10     | 10     | 11    | 25      | 7.9%   | 2.16 (1.38 to 3.39)    |        |
| Nishida 2008      | 22     | 35     | 9     | 30      | 11.1%  | 2.10 (1.15 to 3.33)    |        |
| Kang 2008         | 16     | 25     | 8     | 26      | 9.0%   | 2.08 (1.09 to 3.97)    |        |
| Li 2011           | 11     | 16     | 6     | 16      | 6.9%   | 1.83 (0.90 to 3.74)    |        |
| Xie 2013          | 41     | 60     | 13    | 30      | 19.9%  | 1.58 (1.01 to 2.46)    |        |
| Zeng 2009         | 19     | 20     | 6     | 9       | 9.5%   | 1.43 (0.89 to 2.29)    |        |
| Li 2014           | 11     | 15     | 8     | 15      | 9.2%   | 1.38 (0.78 to 2.41)    |        |

**1.5.1 3 points or more**

| Study or Subgroup | Statin | Events | Total | Control | Events | Total | Weight | Risk Ratio | Subtotal (95% Cl) | Total events |
|-------------------|--------|--------|-------|---------|--------|-------|--------|------------|-------------------|--------------|
| Nozaki 2012       | 4      | 15     | 0     | 23      | 0.5%   | 13.50 (0.78 to 233.96) | 154 51 |
| Iwakiri 2008      | 21     | 30     | 5     | 30      | 5.7%   | 4.20 (1.82 to 9.67)    |        |
| Kang 2007         | 24     | 32     | 5     | 16      | 7.7%   | 2.40 (1.13 to 5.10)    |        |
| Kang 2010         | 39     | 52     | 8     | 24      | 12.6%  | 2.25 (1.25 to 4.05)    |        |
| Cui 1997          | 10     | 10     | 11    | 25      | 7.9%   | 2.16 (1.38 to 3.39)    |        |
| Li 2011           | 11     | 16     | 6     | 16      | 6.9%   | 1.83 (0.90 to 3.74)    |        |
| Zeng 2009         | 19     | 20     | 6     | 9       | 9.5%   | 1.43 (0.89 to 2.29)    |        |

**1.5.2 less than 3 points**

| Study or Subgroup | Statin | Events | Total | Control | Events | Total | Weight | Risk Ratio | Subtotal (95% Cl) | Total events |
|-------------------|--------|--------|-------|---------|--------|-------|--------|------------|-------------------|--------------|
| Kang 2008         | 24     | 32     | 5     | 16      | 7.7%   | 2.40 (1.13 to 5.10)    |        |
| Cui 1997          | 10     | 10     | 11    | 25      | 7.9%   | 2.16 (1.38 to 3.39)    |        |
| Li 2011           | 11     | 16     | 6     | 16      | 6.9%   | 1.83 (0.90 to 3.74)    |        |
| Zeng 2009         | 19     | 20     | 6     | 9       | 9.5%   | 1.43 (0.89 to 2.29)    |        |

**the authors of the studies to collect raw data and complete the search strategy when possible. The two investigators independently selected potential eligible studies according to predetermined criteria (Table I). No language restrictions were imposed. Any discrepancy between them was resolved by consensus. No**
| Study or Subgroup | Statin Events | Control Events | Total Events | Total Weight | Risk Ratio M-H, Fixed, 95% CI |
|-------------------|--------------|----------------|--------------|-------------|-------------------------------|
| **1.7.1 rabbits** |              |                |              |             |                               |
| Iwakiri 2008      | 21           | 30             | 53           | 30          | 4.20 (1.82 to 9.67)           |
| Kang 2007         | 24           | 32             | 56           | 16          | 2.40 (1.13 to 5.10)           |
| Kang 2010         | 39           | 52             | 91           | 24          | 2.25 (1.25 to 4.05)           |
| Nishida 2008      | 22           | 35             | 57           | 9           | 2.10 (1.15 to 3.83)           |
| Kang 2008         | 16           | 25             | 41           | 8           | 2.08 (1.09 to 3.97)           |
| Li 2011           | 11           | 16             | 27           | 6           | 1.83 (0.90 to 3.74)           |
| Zeng 2009         | 19           | 20             | 39           | 9           | 1.43 (0.89 to 2.29)           |
| Li 2014           | 11           | 15             | 26           | 8           | 1.38 (0.78 to 2.41)           |
| Subtotal (95% CI) | 225          | 166            | 391          | 77.9%       | 2.12 (1.68 to 2.66)           |
| **1.7.2 rats**    |              |                |              |             |                               |
| Nozaki 2012       | 4            | 15             | 19           | 5           | 13.50 (0.78 to 233.96)        |
| Xie 2013          | 41           | 60             | 101          | 13          | 1.58 (1.01 to 2.46)           |
| Subtotal (95% CI) | 75           | 53             | 128          | 22.1%       | 1.85 (1.19 to 2.86)           |
| **1.8.12 weeks before** |        |                |              |             |                               |
| Nozaki 2012       | 4            | 15             | 19           | 5           | 13.50 (0.78 to 233.96)        |
| Iwakiri 2008      | 21           | 30             | 51           | 30          | 4.20 (1.82 to 9.67)           |
| Kang 2007         | 24           | 32             | 56           | 16          | 2.40 (1.13 to 5.10)           |
| Kang 2010         | 39           | 52             | 91           | 24          | 2.25 (1.25 to 4.05)           |
| Nishida 2008      | 22           | 35             | 57           | 9           | 2.10 (1.15 to 3.83)           |
| Kang 2008         | 16           | 25             | 41           | 8           | 2.08 (1.09 to 3.97)           |
| Xie 2013          | 41           | 60             | 101          | 13          | 1.58 (1.01 to 2.46)           |
| Subtotal (95% CI) | 249          | 179            | 428          | 66.5%       | 2.26 (1.77 to 2.90)           |
| **1.8.2 simultaneously** |        |                |              |             |                               |
| Cui 1997          | 10           | 10             | 20           | 11          | 1.67 (1.27 to 2.19)           |
| Li 2011           | 11           | 16             | 27           | 6           | 1.83 (0.90 to 3.74)           |
| Zeng 2009         | 19           | 20             | 39           | 6           | 1.43 (0.89 to 2.29)           |
| Li 2014           | 11           | 15             | 26           | 8           | 1.38 (0.78 to 2.41)           |
| Subtotal (95% CI) | 61           | 65             | 126          | 33.5%       | 1.67 (1.27 to 2.19)           |

**Fig. 4**
Forest plot showing subgroup analysis based on species (M-H, Mantel–Haenszel; CI, confidence interval; df, degrees of freedom). Risk ratio (RR) on the left axis indicates statin usage decreases the risk of osteonecrosis compared with the control group, whereas RR greater than 1 indicates animals with statin usage are at increased risk of osteonecrosis. The 95% CI reveals that the result is statistically significant when “1” is not included in the interval, and vice versa.

**Fig. 5**
Forest plot showing subgroup analysis based on treatment time point (M-H, Mantel–Haenszel; CI, confidence interval; df, degrees of freedom). Risk ratio (RR) on the right axis indicates statin usage decreases the risk of osteonecrosis compared with the control group, whereas RR greater than 1 indicates animals with statin usage are at increased risk of osteonecrosis. The 95% CI reveals that the result is statistically significant when “1” is not included in the interval, and vice versa.
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A distinction was made for animal models and the type or dose range of statins and GCs.

**Data extraction.** Data collection was conducted by two investigators independently and the result was checked by a third investigator. Discrepancies were settled by group discussion. Collected data included the first author’s surname; publication year; study location; study design; sample size; type or dose range of statins and GC; duration of GC and statin usage; characteristics of animal models including species, animal age, weight, gender and route of delivery in which GC and statins were administered; the absolute number of ON cases in groups with or without statin exposure and the total number of animals with or without statin usage. When various statins or dosages were present in one study, data were analysed as a single group of those exposed to statins.

**Assessment of methodological quality.** Two reviewers independently assessed the methodology of the included articles with use of the updated Stroke therapy Academic Industry Roundtable (STAIR) recommendations. The methodological quality of individual study was scored against the following criteria: sample size calculation; inclusion and exclusion criteria; randomisation; allocation concealment; reporting of animals excluded from analysis; blinded assessment of osteonecrosis; reporting potential conflicts of interest and study funding. Each item was allocated one point for a quantitative appraisal of overall quality of the individual studies. Each study was given a quality score out of a possible total of seven points, and the group median was calculated.

**Statistical analysis.** The risk ratio (RR) was calculated by two investigators using the Cochrane review manager software (Version 5.3, Cochrane Collaboration, Oxford, United Kingdom). RR was calculated along with 95% confidence intervals (CI). Assessment of heterogeneity of included studies was conducted using Q and I² statistics.

### Table: Risk Ratio and Subgroup Analysis

| Study or Subgroup | Statin Events | Control Events | Total Events | Risk Ratio | Risk Ratio |
|-------------------|---------------|----------------|--------------|------------|------------|
|                   | Total | % | % | Total | % | % | M-H, Fixed, 95% CI | M-H, Fixed, 95% CI |
| 1.9.1 histological examination | | | | | | | |
| Nozaki 2012 | 4 | 20 | 23 | 0.6% | 13.50 (0.78 to 233.96) | | |
| Cui 1997 | 8 | 3 | 25 | 2.5% | 6.67 (2.21 to 20.14) | | |
| Kang 2007 | 24 | 32 | 5 | 16 | 9.6% | 2.40 (1.13 to 5.10) | | |
| Iwakiri 2008 | 21 | 30 | 9 | 30 | 12.9% | 2.33 (1.29 to 4.23) | | |
| Kang 2010 | 39 | 52 | 8 | 24 | 15.7% | 2.25 (1.25 to 4.05) | | |
| Nishida 2008 | 22 | 35 | 9 | 30 | 13.9% | 2.10 (1.15 to 3.83) | | |
| Kang 2008 | 16 | 25 | 8 | 26 | 11.9% | 2.08 (1.09 to 3.97) | | |
| Li 2011 | 11 | 16 | 6 | 16 | 8.6% | 1.83 (1.90 to 3.74) | | |
| Xie 2013 | 41 | 60 | 13 | 30 | 24.9% | 1.58 (1.01 to 2.46) | | |
| Subtotal (95% CI) | 275 | 220 | 100% | 2.20 (1.77 to 2.75) | | |
| Total events | 186 | 61 | | | | |
| Heterogeneity: Chi² = 7.99, df = 8 (p = 0.43); I² = 0% | | | | | |

Test for overall effect: Z = 7.06 (p < 0.00001)

### Subgroup Analysis

| 1.9.2 x-ray examination |
|-------------------------|
| Zang 2009 | 19 | 20 | 6 | 9 | 50.8% | 1.43 (0.89 to 2.29) | | |
| Li 2014 | 11 | 15 | 8 | 15 | 49.2% | 1.38 (0.78 to 2.41) | | |
| Subtotal (95% CI) | 35 | 24 | 100% | 1.40 (0.87 to 2.02) | | |
| Total events | | | | | | |
| Heterogeneity: Chi² = 0.01, df = 1 (p = 0.92); I² = 0% | | | | | |

Test for overall effect: Z = 1.80 (p = 0.07)

Test for subgroup differences: Chi² = 4.34, df = 1 (p = 0.04); I² = 77.0%

Forest plot showing subgroup analysis based on measurement (M-H, Mantel–Haenszel; CI, confidence interval; df, degrees of freedom). Risk ratio (RR) on the left axis indicates statin usage decreases the risk of osteonecrosis compared with the control group, whereas RR greater than 1 indicates animals with statin usage are at increased risk of osteonecrosis. The 95% CI reveals that the result is statistically significant when “1” is not included in the interval, and vice versa.

Funnel plots evaluating publication bias among included studies.
Heterogeneity was not considered present with a p-value ≥ 0.1. Subgroup analyses were performed where applicable. Publication bias was accessed by funnel plots, using Egger’s tests.25 Significance was considered when a p-value was < 0.05.

Results

Search results and study characteristics. According to the aforementioned specific criteria, 11 eligible studies,13-18,23,26-29 published between 1997 and 2014 were included in our meta-analysis. Figure 1 shows the detailed process of study selection. In total, we included 554 animals consisting of 391 rabbits, 128 rats and 35 chickens. Animal model ON was induced using steroid alone in all studies except for one,23 which combined steroid with endotoxin. The study conducted by Nozaki et al13 consisted of rats with a 50% incidence of spontaneous ON of the femoral head at the age of 16 to 18 weeks, while the remaining studies consisted of healthy animals. Characteristics of the included studies are summarised in Tables II to VII. The median of the reported quality score was 3 (2 to 5).

Primary analysis. The overall estimate of the effect of statins was 2.06 (95% CI 1.71 to 2.50, p < 0.001), an approximately two-fold improvement in outcome (Fig. 2). Compared with the control group, the RR was 1.91 (95% CI 1.50 to 2.42) in studies using statins alone while the RR was 2.10 (95% CI 1.68 to 2.62) in studies using statins with other interventions such as bisphosphonate or anticoagulant. Then we further compared the effect of statin exposure alone to that of statins combined with other interventions using data from experiments containing both groups. Results revealed that combined usage significantly decreased the risk of ON (RR = 1.23, 95% CI, 1.02 to 1.47). However, we could find no significant risk difference for different gender or for different treatment points (two weeks before or simultaneously with GC injection).

The overall risk estimate of statins in the present study supports the findings of previous work conducted by Pritchett11 who found that only three patients (3/284, 1%) treated with statins developed ON, much less than the 3% to 20% incidence usually reported for patients receiving high-dose steroids. However, our findings do not correlate with those of the cohort study by Ajmal et al,10 in which 2881 renal transplantation patients were evaluated and 4.4% (15/338) of patients on statins developed ON compared with 7% (180/2543) of patients who were not on statins. The authors concluded that statin usage does not appear to lower the risk of ON. There are several possible explanations for the discrepancy in the results. Firstly, the dosage of GC administrated to patients following renal transplantation was uncertain at baseline. Higher doses, even of short duration, present greater risks.2 Secondly, the indication for statin treatment was confined to patients with hypercholesterolaemia. And thirdly, the measure of ON was not reported and it is also unknown whether the measurement was taken identically for each patient.

In a randomised double-blinded placebo-controlled study, Lydonet, Schweitzer and Belmont30 also found that the incidence of newly developed ON in patients with 40 mg daily of atorvastatin was not statistically different to that found in those administered hydroxychloroquine or bisphosphonates. However, it must be noted that the patients in the control group were administrated bisphosphonates, which may reduce the risk of developing ON to some degree.4,26,28

Our results also revealed that statin usage in combination with bisphosphonate or anticoagulant significantly decreased the risk of ON, compared with statins alone, suggesting a protective role for bisphosphonates or anticoagulants against developing GC-induced ON. These findings have been supported elsewhere in the literature.14,20,28,29,31

In this current study, we could find no significant difference in risk between animal gender. This differs from the published studies conducted by Ajmal et al,10 who found that male gender is associated with an increased risk (34%) of ON compared with female patients. The reason for this finding is not clear but it may be related to different mechanisms of fat metabolisms between genders.32,33

With regard to animal species, risk estimate was lower in rabbits than in rats. To our knowledge, this has not been reported previously. A possible explanation for this might be that animal models used in Nozaki et al’s study13
consist of rats with a 50% incidence of spontaneous ON of the femoral head, rather than rats in healthy condition.

Both of the intervention protocols (statin exposure started at two weeks before versus simultaneously with methylprednisolone injection) demonstrated protective action against development of ON, but there was no significant difference in effect size between the two groups. To the best of our knowledge, this comparison has not been reported previously. A possible explanation for this finding might be that different statin dosage and species were used across studies. It also may be due to a possible threshold effect for development of ON.14,29

As for assessment of ON, it was more conservatively estimated with the use of radiographic examination than with the use of histological examination. Our findings are consistent with those of Kang et al34 who found no significant changes of ON examined using radiographs during two to 12-week periods. Accordingly, the difference in risk estimate between various measurements could be explained by the lower detection rate of radiographic examination. Given the small sample size of animals examined using radiographs, however, the results should be considered with caution.

Several limitations must be considered in interpreting our findings. First, the time point at which the outcome was measured varied from study to study. However, ON was evaluated in all included studies at a time point more than two weeks after steroid injection, since two weeks is a time point that has been reported to be crucial in the development of ON.7,16 Secondly, the sites measured for diagnosis of ON were not controlled for all included studies. The more sites per animal evaluated, the greater risk for the detection of ON. Thirdly, effect size might be weakened in animals with comorbidities. Finally, the preventive effects of different statin dosage, and various types of statins on the development of ON, were not analysed.

In conclusion, the present study revealed that statin usage could reduce the risk of GC-related ON in animal models, suggesting statins are efficacious in preventing GC-induced ON to some degree in animals. However, while the overall results of this meta-analysis suggest that statins have substantial effect size, concerns should be raised that the true efficacy of statins might be substantially lower than reported here because of the possible influence of publication or other bias. Furthermore, given the limited application of experimental studies to humans, the heterogeneity among studies and lack of high quality evidence, the results should be extrapolated to the clinical setting with great caution.

Supplementary material
A table showing the search strategy is available alongside this article at www.bjr.boneandjoint.org.uk

References
1. Malizos KN, Karantanas AH, Varitimidis SE, et al. Osteonecrosis of the femoral head: etiology, imaging and treatment. Eur J Radiol 2007;63:16-28.
2. Parsons SJ, Steele N. Osteonecrosis of the femoral head: Part 1—Aetiology, pathogenesis, investigation, classification. Curr Orthop 2007;21:457-463.
3. Assouline-Dayan Y, Chang C, Greenspan A, Shoenfeld Y, Gershwin ME. Pathogenesis and natural history of osteonecrosis. Semin Arthritis Rheum 2002;32:94-124.
4. Parsons SJ, Steele N. Osteonecrosis of the femoral head: Part 2 Options for treatment. Curr Orthop 2008;22:349-358.
5. Fukushima W, Fujioka M, Kubo T, et al. Nationwide epidemiologic survey of idiopathic osteonecrosis of the femoral head. Clin Orthop Relat Res 2010;468:2715-2724.
6. Weinstein RS. Clinical practice. Glucocorticoid-induced bone disease. N Engl J Med 2011;365:62-70.
7. Miyaniishi K, Yamamoto T, Irima T, et al. Bone marrow fat cell enlargement and a rise in intraosseous pressure in steroid-treated rabbits with osteonecrosis. Bone 2002;30:185-190.
8. Ichikawa T, Matsumoto T, Nishino M, Kaneuji A, Katsuda S. Oxidative stress and vascular permeability in steroid-induced osteonecrosis model. J Orthop Sci 2004;9:509-515.
9. Jiang Y, Zhang Y, Zhang H, et al. Pravastatin prevents steroid-induced osteonecrosis in rats by suppressing PPARγ expression and activating Wnt signaling pathway. Exp Biol Med (Maywood) 2014;239:347-355.
10. Ajmal M, Matas AJ, Kuskowski M, Cheng EY. Does statin usage reduce the risk of corticosteroid-related osteonecrosis in renal transplant population? Orthop Clin North Am 2009;40:235-239.
11. Pritchett JW. Statin therapy decreases the risk of osteonecrosis in patients receiving steroids. Clin Orthop Relat Res 2001;386:173-178.
12. Belmont HM, Lydon E. Avascular necrosis prevention with ligator in lupus erythematosus. Lupus 2005;14:869-870.
13. Nozaki Y, Kumagai K, Miyata N, Niwa M. Pravastatin reduces steroid-induced osteonecrosis of the femoral head in SHRS rats. Acta Orthop 2012;83:87-92.
14. Kang P, Gao H, Pei F, et al. Effects of an anticoagulant and a lipid-lowering agent on the prevention of steroid-induced osteonecrosis in rabbits. Int J Exp Pathol 2010;91:235-242.
15. Iwakiri K, Oda Y, Kaneshiro Y, et al. Effect of simvastatin on steroid-induced osteonecrosis evidenced by the serum lipid level and hepatic cytochrome P4503A in a rabbit model. J Orthop Sci 2008;13:463-468.
16. Pengde K, Fuxing P, Bin S, Jing Y, Jingqiu C. Lovastatin inhibits adipogenesis and prevents osteonecrosis in steroid-treated rabbits. J Bone Spine 2008;75:696-701.
17. Nishida K, Yamamoto T, Motomura G, Jingushi S, Iwamoto Y. Pitavastatin may reduce risk of steroid-induced osteonecrosis in rabbits: a preliminary histological study. Clin Orthop Relat Res 2008;466:1054-1059.
18. Cui Q, Wang GJ, Su CC, Balan G. The Otto Award. Lovastatin prevents steroid-induced adipogenesis and osteonecrosis. Clin Orthop Relat Res 1997;344:S-19.
19. Wang GJ, Rawltes JG, Hubbard SL, Stamp WG. Steroid-induced femoral head pressure changes and their response to lipid-clearing agents. Clin Orthop Relat Res 1983;174:298-302.
20. Motomura G, Yamamoto T, Miyaniishi K, Jingushi S, Iwamoto Y. Combined effects of an anticoagulant and a lipid-lowering agent on the prevention of steroid-induced osteonecrosis in rabbits. Arthritis Rheum 2004;50:3387-3391.
21. Jasińska M, Owczarek J, Orszulak-Michalak D. Statins: a new insight into their mechanisms of action and consequent pleiotropic effects. Pharmacol Rep 2007;59:483-499.
22. Vaughan CJ, Murphy MB, Buckley BM. Statins do more than just lower cholesterol. Lancet 1999;348:1079-1082.
23. Li TS, Xiao ZM, Huang OP, et al. Impact of lipid lowering and anticoagulant combined application on the femoral bone cell apoptosis in the rabbits with steroid-induced necrosis. Journal of China Medical University 2014;43:441-445.
24. Fisher M, Feuerstein G, Howells DW, et al. Update of the stroke therapy academic industry roundtable preclinical recommendations. Stroke 2009;40:2244-2250.
25. Egger M, Davey Smith G, Schneider M, Minder C. Bias in meta-analysis detected by a simple, graphical test. BMJ 1997;315:629-634.
26. Zeng XJ, Yang SH, Yu ZH. Preventive effects of Simvastatin combined alendronate on steroid-induced osteonecrosis of the femoral head in rabbits. J YMC 2009;28:237-239.
27. Li TS, Xiao ZM, Zhan XL. Effects of pravastatin combined with warfarin on steroid-induced femoral head necrosis in rabbits. Chinese Journal of Pathophysiology 2011;27:1603-1608.
28. Xie X, Kang P, Pei F, et al. Effect of alendronate and lovastatin in preventing early glucocorticoids-induced osteonecrosis of femoral head in rats by micro-CT. *Orthopedic Journal of China* 2013;21:82-86.

29. Kang P, Shen B, Yang J, et al. Prevention of steroids-induced osteonecrosis of the femoral head with anticoagulant and lipid-lowering management in rabbits. *Chin J Joint Surg* 2007;30:58-61.

30. Lydon E, Schweitzer M, Belmont HM. Atorvastatin to prevent avascular necrosis of bone in systemic lupus erythematosus. *Arthritis Rheum* 2006;54:5432.

31. Yamamoto T, Miyanishi K, Motomura G, et al. Animal models for steroid-induced osteonecrosis. *Clin Calcium* 2007;17:879-886.

32. Mittendorfer B. Sexual dimorphism in human lipid metabolism. *J Nutr* 2005;135:681-686.

33. Lima JJ, Mauras N, Kissoon N, et al. Influence of sex and beta2 adrenergic receptor haplotype on resting and terbutaline-stimulated whole body lipolysis. *Metabolism* 2005;54:492-499.

34. Kang P, Yang J, Shen B, et al. An experimental dynamic study on the relationship between histopathological and radiological changes in steroid-induced femoral head osteonecrosis of rabbits. *Chin J Orthop* 2010;30:92-97.

Funding Statement
- This work was supported by the China Health Ministry Program (Grants 201302007), the National Natural Science Fund of China (Grants 81271786/810605 and 81171763) and the Sichuan Province Science and Technology Support Program (Grant 2013010151).

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ICMJE conflict of interest
- None declared

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