

1           **Is there a role for vitamin C in preventing osteoporosis and fractures? A review of the**  
2           **potential underlying mechanisms and current epidemiological evidence**

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4   **Authors:**

5   Henriette Finck, Andrew R. Hart, Amy Jennings and Ailsa A. Welch

6  
7   **Institution:**

8   Norwich Medical School, University of East Anglia, Norwich, NR4 7TJ

9  
10   **Corresponding author:**

11   Dr Ailsa Welch, Norwich Medical School, University of East Anglia, Norwich, NR4 7TJ, tel 01603  
12   591950, email [a.welch@uea.ac.uk](mailto:a.welch@uea.ac.uk)

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19 **Abstract**

20 Osteoporosis and related fractures are a major global health issue, but there are few preventative  
21 strategies. Previously reported associations between higher intakes of fruits and vegetables and  
22 skeletal health have been suggested to be partly attributable to vitamin C. To date, there is some  
23 evidence for a potential role of vitamin C in osteoporosis and fracture prevention but an overall  
24 consensus of published studies has not yet been drawn. This review aims to provide a summary of  
25 the proposed underlying mechanisms of vitamin C on bone and reviews the current evidence in the  
26 literature, examining a potential link between vitamin C intake and status with osteoporosis and  
27 fractures. The Bradford Hill criteria were used to assess reported associations. Recent **animal** studies  
28 have provided insights into the involvement of vitamin C in osteoclastogenesis and  
29 osteoblastogenesis; and **its role** as a mediator of bone matrix deposition, affecting both the quantity  
30 and quality of bone collagen. Observational studies have provided some evidence for this in the  
31 general population showing positive associations between dietary **vitamin C** intake and supplements  
32 and higher bone mineral density or reduced fracture risk. However, previous intervention studies were  
33 not sufficiently well designed to evaluate these associations. Epidemiological data are particularly  
34 limited for vitamin C status and for fracture risk and good quality RCTs are needed to confirm  
35 previous epidemiological findings. This review also **highlights** that associations between vitamin C  
36 and bone health may be non-linear and further research is needed to ascertain optimal intakes for  
37 osteoporosis and fracture prevention.

## 38 **Introduction**

39 Osteoporosis is a “progressive systemic skeletal disease characterised by low bone mass and  
40 microarchitectural deterioration of bone tissue, with a consequent increase in bone fragility and  
41 susceptibility to fracture”<sup>(1)</sup>. The condition has been estimated to affect 75 million people in Europe,  
42 Japan and the United States<sup>(2)</sup>. Moreover, fragility fractures, the clinical manifestation of  
43 osteoporosis, are a major global health issue with an annual prevalence of 8.9 million fractures  
44 worldwide<sup>(3)</sup>. The elderly are the most at-risk population<sup>(4)</sup> and as the world’s population aged 60 and  
45 80 years plus is estimated to increase three and seven fold by 2100, respectively<sup>(5)</sup>, osteoporosis and  
46 related fractures will become an increasingly bigger health burden.

47 Risk factors for the development of osteoporosis and fragility fractures include genetic and  
48 biological factors, although environmental factors, including diet, are of great interest for developing  
49 preventative strategies, as they are modifiable. To date, a wide range of nutrients, foods and food  
50 groups have been studied in relation to bone health, including fruits and vegetables with every  
51 increased serving or intakes of 1-4 portions per day, on at least three days per week, being positively  
52 associated with increased bone mass or a reduction in bone loss<sup>(6-9)</sup>. The mechanisms underlying these  
53 positive associations have not been fully elucidated but one such explanation is the potential buffering  
54 effect of the overall dietary acid load constituents in fruits and vegetables<sup>(10)</sup>. **Moreover,**  
55 **epidemiological studies have suggested that these beneficial effects may also be due to micronutrients**  
56 **such as vitamin C which may have mechanisms independent of these buffering effects**<sup>(11,12)</sup>. Vitamin  
57 C, an essential nutrient to humans found in citrus and soft fruits<sup>(13,14)</sup>, has previously been linked to  
58 bone health, particularly bone structure. For example, in previous animal studies vitamin C  
59 deprivation resulted in a marked reduction in bone formation<sup>(15-17)</sup>; and superoxide-induced bone loss  
60 in mice was restored by oral administration of 1% vitamin C in drinking water, as evidenced by  
61 significant improvements in BMD, bone weight, bone strength and collagen cross-links<sup>(18)</sup>. In the last  
62 two decades, observational and intervention studies have investigated a potential role for vitamin C  
63 in osteoporosis and fracture prevention; however, an overall consensus of the results of published  
64 studies does not exist.

65 This article provides a review of the potential underlying mechanisms of vitamin C in bone  
66 metabolism. The current evidence in the literature investigating a potential role for vitamin C in the  
67 prevention of osteoporosis and related fractures will be discussed and avenues for future research  
68 highlighted. Databases, including MEDLINE (Ovid), PubMed and Google Scholar, were used to  
69 identify relevant observational and clinical studies published up to August 2013. As neither laboratory  
70 nor epidemiological studies can infer causality, criteria established by Sir Austin Bradford Hill in  
71 1965 were used to assess whether vitamin C is causal in the prevention of osteoporosis and associated  
72 fractures<sup>(19)</sup>. The structure of the review will be discussed around these criteria.

73

**74 Bradford Hill criteria**

75 The Bradford Hill criteria (BHC) are a set of guidelines used to assess causality of hypotheses and  
76 associations from trial, laboratory and epidemiological research<sup>(19)</sup>. In brief, the nine criteria assess  
77 (1) biological plausibility, (2) coherence between laboratory and epidemiological studies, (3)  
78 temporality, (4) consistency, (5) strength, (6) analogy, (7) specificity, (8) dose-response effect, and  
79 (9) evidence from intervention studies. The criteria may not confirm the absence or presence of  
80 causality unconditionally, but are considered to be a useful tool for understanding associations  
81 between an exposure and a risk of disease.

82

**83 Potential mechanisms of vitamin C in bone health**

84 Scurvy, the clinical manifestation of vitamin C deficiency, is associated with wounds and fractures  
85 that fail to heal. The discovery of vitamin C in the early 20<sup>th</sup> century and subsequent animal studies  
86 lead to the suggestion that scurvy symptoms result from impaired collagen formation in vitamin C  
87 deficiency<sup>(20)</sup>. Collagen is an essential component of bone tissue; and more recently, many cell and  
88 animal studies reported that vitamin C may also mediate osteoclastogenesis and osteoblastogenesis<sup>(21-</sup>  
89 <sup>24)</sup>, although the precise biological mechanisms have not been fully established yet.

90

**91 Osteoclastogenesis**

92 Vitamin C has been suggested to mediate osteoclast differentiation and possibly apoptosis<sup>(22,25)</sup> and  
93 findings have been relatively consistent. In cell cultures containing both osteoblasts and osteoclasts,  
94 vitamin C promoted osteoclastogenesis<sup>(26-28)</sup> and this was associated with an increase in RANKL  
95 expression<sup>(27)</sup>. In concordance with these findings, vitamin C deficiency resulted in a decrease in  
96 osteoclast differentiation<sup>(26,27)</sup>. However, in cultures containing only osteoclasts, stimulatory  
97 effects<sup>(29)</sup> as well as inhibitory effects<sup>(22,28,30)</sup> of vitamin C on osteoclast differentiation have been  
98 reported. Recent *in vitro* findings have helped explain these contradictory results by showing that  
99 vitamin C at a concentration of 50 µg/ml initially exhibited pro-oxidant activity resulting in an  
100 increase in the number, size and nucleation of osteoclasts, although vitamin C also initiated  
101 accelerated osteoclast death at later stages<sup>(25)</sup>. Deficiency studies are in agreement with most previous  
102 findings, indicating that vitamin C deficiency in animal models stimulated osteoclastogenesis via the  
103 up-regulation of the RANKL/RANK pathway<sup>(22,23)</sup>. Moreover, **vitamin C deficient** mice  
104 supplemented with vitamin C had a reduction in RANKL expression<sup>(23)</sup>. Although there is some  
105 consistency of previous cell and animal studies reporting on the effects of vitamin C on  
106 osteoclastogenesis, the current discrepancies require further investigation in humans to help decide if  
107 vitamin C may be involved in osteoclastogenesis via mediating the RANK/RANKL pathway.

108

**Osteoblastogenesis**

110 Vitamin C may be involved in accentuating osteoblastogenesis. For example, a decrease in the  
111 number of osteoblasts and suppressed osteoblast differentiation has previously been observed in  
112 vitamin C deficient mice<sup>(23)</sup>. In concordance with these findings, an increase in the number of  
113 osteoblasts following vitamin C treatment has been reported from *in vitro* work<sup>(31)</sup>. Furthermore,  
114 studies using osteoblast-like cell cultures including human tissue have shown that osteoblast  
115 proliferation and differentiation was enhanced with the addition of vitamin C<sup>(21,24,31-33)</sup>.  
116 Concentrations of 50 µg/ml and 200 µg/ml vitamin C have previously been suggested as optimal and  
117 maximum concentrations for this effect<sup>(21,24)</sup>.

118 Initially, work suggested that the effects of vitamin C on osteoblastogenesis may be through  
119 stimulating collagen synthesis<sup>(31,32)</sup>, although more recent evidence suggests the underlying  
120 mechanisms are more complex. For example, vitamin C has been reported to mediate gene expression  
121 of a number of genes involved in pre-osteoblast cell activities including growth, metabolism,  
122 communication and death<sup>(34)</sup>. Furthermore, **animal** studies have shown that the expression of PPAR-  
123  $\gamma$  may mediate osteoblast differentiation resulting in bone loss<sup>(35,36)</sup>. Recently, these findings have  
124 been investigated further and a link to vitamin C **has been** established. An *in vivo* study reported that  
125 PPAR- $\gamma$  expression in osteoblasts was significantly up-regulated in vitamin C deficient mice and was  
126 accompanied by suppressed osteoblast differentiation; whereas treatment with vitamin C mediated  
127 PPAR- $\gamma$  expression to almost normal levels<sup>(23)</sup>. To date, there is consistent experimental evidence for  
128 a beneficial role of vitamin C in osteoblastogenesis. Recent work suggesting that vitamin C may  
129 mediate PPAR- $\gamma$  expression has provided more insight **in to the mechanisms**, and further  
130 experimental studies are needed to confirm these findings.

131

**Bone collagen synthesis**

133 Vitamin C is essential for collagen type I synthesis by osteoblasts. For example, early *in vitro* work  
134 reported that collagen synthesis increased more than four fold in the presence of ascorbate<sup>(37)</sup> and  
135 more recently, greater amounts of collagen were shown to be present at vitamin C concentrations of  
136 200 µg/ml compared to 100 µg/ml and 25 µg/ml<sup>(24)</sup>. The underlying mechanisms for this are thought  
137 to relate to the role of vitamin C in stimulating collagen synthesis and as a cofactor of hydroxylation  
138 reactions within collagen fibres. For the former, vitamin C is an important initiator of collagen  
139 synthesis in osteoblasts<sup>(38)</sup>, possibly via stimulating pro-collagen type I mRNA<sup>(39,40)</sup>; whereas for the  
140 latter, vitamin C is an essential activator of enzymes involved in the hydroxylation of proline and  
141 lysine residues within collagen fibres<sup>(41-44)</sup>. The hydroxylation reaction enables the formation of  
142 covalent bonds between the amino acid residues, increasing overall collagen strength. Early *in vitro*

143 and *in vivo* studies found that the lack of ascorbic acid resulted in the formation of underhydroxylated  
144 and unhydroxylated collagen<sup>(45-49)</sup>, thus decreasing bone matrix stability and weakening bone  
145 structure. In contrast, the presence of vitamin C increased the hydroxylation of amino acid residues  
146 *in vitro*<sup>(50)</sup>. The hydroxylation of amino acid residues may occur while the collagen polypeptide chain  
147 is still being synthesised and attached to the ribosome<sup>(51,52)</sup>. However, more recent work suggested  
148 that this hydroxylation reaction takes place in the endoplasmic reticulum<sup>(53)</sup>.

149 Experimental evidence for a role of vitamin C in bone collagen synthesis is well established.  
150 Vitamin C is important for the quality of collagen via its cofactor role in hydroxylation reactions in  
151 collagen fibres. Future studies should focus on the importance of vitamin C for the quantity of  
152 collagen synthesis via stimulating procollagen type I mRNA as there are currently only limited data  
153 on this potential link.

154

155 In summary, a range of mechanisms of vitamin C in maintaining bone health have been suggested in  
156 a number of experimental studies. Thus, there is some good evidence for the BHC of biological  
157 plausibility for vitamin C deficiency and osteoporosis. The evidence for a role of vitamin C in  
158 osteoblastogenesis and in quality aspects of bone collagen synthesis is consistent. In contrast, **the**  
159 **links** between vitamin C and osteoclastogenesis as well as quantity aspects of collagen synthesis are  
160 currently less well defined and require further investigation.

161

## 162 **Measures of vitamin C intake and status**

163 Vitamin C intake may be measured from dietary assessment methods such as food diaries and  
164 FFQs<sup>(54)</sup>. Food diaries assess habitual intake through a detailed description of foods and drinks  
165 consumed typically in the preceding **three to** seven days and FFQs make use of a food list with a  
166 frequency response section estimating intake usually from the previous 12 months. The mean vitamin  
167 C intake in the UK is 90 mg/d (calculated using food records)<sup>(55)</sup>, reflecting sufficient intake  
168 according to the **Reference Nutrient Intake (RNI)** of 40 mg/d<sup>(13)</sup> and in comparison to the US  
169 recommendations of 90 mg/d and 75 mg/d for men and women, respectively<sup>(56)</sup>. The Lower Reference  
170 Nutrient Intake (LRNI) has been set in the UK at 10 mg/d and is based on the prevention and cure of  
171 scurvy<sup>(13)</sup>. Currently, there is no upper limit for vitamin C intake. However, very high intakes of 1000  
172 mg/d and above, achieved through the use of supplements, may present with side effects including  
173 gastrointestinal discomfort and diarrhoea<sup>(57)</sup> **and have previously been shown to increase the risk of**  
174 **renal stones**<sup>(58)</sup>.

175 The ability to accurately assess vitamin C intake varies between the different dietary methods,  
176 with **the** correlation coefficients between blood vitamin C concentrations and dietary intake being  
177 higher for food diaries, dietary recalls (**both**  $r$  0.46; 95% CI 0.41, 0.52) and weighed records ( $r$  0.39;

178 95% CI 0.25, 0.53) compared to the correlation coefficient between blood vitamin C concentrations  
179 and dietary intake estimated from FFQs ( $r$  0.35; 95% CI 0.29, 0.40)<sup>(54)</sup>. Despite the ability to estimate  
180 vitamin C intake, the measurement of vitamin C status from blood may be more accurate than dietary  
181 intake assessments as it avoids human recall error and variations in individual bioavailability of the  
182 nutrient and accounts for factors that affect the vitamin C composition of food including length of  
183 storage of food items and cooking practises<sup>(59)</sup>. However, vitamin C in blood is influenced by a  
184 number of biological and lifestyle factors including age<sup>(60)</sup>, sex<sup>(61,62)</sup>, BMI<sup>(60)</sup>, body fat distribution<sup>(63)</sup>,  
185 smoking<sup>(64,65)</sup> and infection<sup>(66)</sup> which should be accounted for when evaluating its association with  
186 disease risk.

187 Dietary intake and plasma concentrations of vitamin C, when plotted against each other, show  
188 a sigmoidal relationship<sup>(67,68)</sup>. Average vitamin C intakes (60-100 mg/d) reflect plasma levels of  
189 around 40-60  $\mu\text{M/l}$ . Higher intakes result in a progressive flattening of the curve and very high intakes  
190 of 400 mg/d and above appear to saturate vitamin C in plasma at concentrations of 70-85  $\mu\text{mol/l}$ ,  
191 leading to the excretion of the vitamin<sup>(68)</sup>. The mean plasma vitamin C concentration of the general  
192 UK population is 53  $\mu\text{mol/L}$ <sup>(69)</sup>. Vitamin C status may be categorised as severely deficient at plasma  
193 levels below 11  $\mu\text{mol/L}$  indicating biochemical depletion; and 1% of men and 2% of women in the  
194 UK are classified as such<sup>(69)</sup>.

## 195

### 196 **Current evidence on vitamin C, osteoporosis and fracture prevention**

197 There is evidence from epidemiological studies for a potential role of vitamin C in maintaining  
198 different aspects of bone health, although the results have varied between studies. In the next section,  
199 randomised controlled trials (RCTs) as the best indicator of causality will be discussed first and this  
200 will be followed by observational studies in hierarchical order of decreasing ability to determine  
201 causality. All types of studies will be evaluated against the BHC.

### 202

#### 203 *Intervention studies*

204 RCTs are the only studies that can definitively infer causality and determine factors influencing  
205 disease, making them the gold standard in limiting selection bias and confounding. To our knowledge,  
206 there is only one such published RCT with a double-blind design that has examined the effects of  
207 vitamin C supplementation on indicators of bone health (Table 1). The study involving 30 men and  
208 women compared bone density of one group taking a placebo with that of two groups receiving 400  
209 IU of vitamin E daily and either 500 mg/d or 1000 mg/d of vitamin C for 12 months<sup>(70)</sup>. The group  
210 with the highest vitamin C intake had significantly less hip bone loss compared to the placebo group  
211 (effect sizes and P-values not shown), although no such observations were made at the lumbar spine.  
212 However, this study did not investigate the effects of vitamin C independently and the inclusion



213 criteria allowed for smokers and for participants with controlled chronic disease which may have  
214 biased the study outcomes. Thus, it remains unclear to what extent vitamin C was involved in  
215 preventing bone loss in this study.

216 Two intervention studies used a combination of an exercise programme and supplementation  
217 with vitamin C and E<sup>(71,72)</sup>. The first study was a randomised placebo-controlled pilot study in 34  
218 women who followed an intervention of 60 minutes of resistant training three times per week and  
219 daily supplementation with vitamin C (1000 mg/d) and E (600 mg/d) for six months. Women were  
220 randomised into four treatment groups of placebo, vitamins, exercise and placebo, or exercise and  
221 vitamins<sup>(72)</sup>. BMD of the lumbar spine but not the femoral neck decreased significantly by 1% in the  
222 placebo group over six months (BMD pre:  $1.01 \pm 0.17$  g/cm<sup>2</sup>; BMD post:  $1.00 \pm 0.16$  g/cm<sup>2</sup>;  $P < 0.05$ )  
223 and was maintained in the other groups. No additive effects of the exercise intervention and the  
224 vitamin supplementation were found. However, the results may have been biased by changes in  
225 dietary habits as a reduction in vitamin C intake over the course of the study period was reported for  
226 the vitamin intervention group. Moreover, the study did not report on blinding in the protocol. The  
227 second study, a two month intervention in 13 men and women, included an hour of aerobic exercise  
228 three times per week and the daily use of vitamin C (500 mg/d) and vitamin E (100 mg/d) supplements  
229 for all subjects<sup>(71)</sup>. Although markers of calcium homeostasis improved significantly (effect sizes not  
230 reported), the bone formation marker BSALP decreased unexpectedly by 14.5% ( $P$ -value not  
231 reported). **However, this study lacked a control group, was undertaken in only 13 individuals, and**  
232 **since it was a mixed intervention, the effects of vitamin C could not be distinguished.** Moreover, both  
233 studies were of short duration of only two to six months, although changes in BMD are more likely  
234 to be observed after a longer duration of treatment.

235 In summary, evidence from current trials investigating potential preventative effects of  
236 vitamin C in osteoporosis remains equivocal, even though the doses were greater than with diet alone.  
237 There are limitations regarding study design, inclusion and exclusion criteria, limited duration of  
238 treatment, small sample sizes and dietary intake that was not controlled for. Moreover, published  
239 intervention studies have used vitamin supplements containing vitamin E in addition to vitamin C  
240 and have included exercise programmes during treatment. Future trials should consider having more  
241 participants, stricter inclusion and exclusion criteria and interventions consisting of vitamin C  
242 supplementation only. The BHC of evidence from intervention studies is therefore not met.

243

#### 244 ***Prospective and longitudinal studies***

245 Prospective cohort studies may be used to investigate the aetiology of a disease as the exposure is  
246 measured prior to the condition occurring, making studies less prone to recall bias than case-control  
247 studies. They may thus also be used to evaluate the BHC of temporality. Furthermore, as cases and



248 controls are drawn from the same population, there is less selection bias. To date, only one prospective  
249 and two longitudinal studies have investigated potential vitamin C and bone associations (Table 2).  
250 One study of 944 men and women from the UK with a mean age of 72 years reported significantly  
251 less total hip BMD loss of up to 54% for higher dietary intakes of vitamin C (99-363 mg/d) compared  
252 to lower intakes (7-57 mg/d)<sup>(73)</sup>. Another study using a US cohort of 606 subjects with a mean age of  
253 75 years reported that lumbar spine and trochanter BMD loss, but not femoral neck and radial shaft  
254 BMD loss, decreased significantly across tertiles of dietary vitamin C intake in men but not in  
255 women<sup>(74)</sup>. However, as highlighted above, the findings were not consistent across these two studies  
256 with results varying mainly for gender and bone site. Potential explanations for this might be that the  
257 first study used 7-day food diaries and did not adjust for important confounders including age, gender  
258 and smoking<sup>(73)</sup>, in contrast to the second study which used a semiquantitative FFQ and measured BMD  
259 via two different types of bone scans (i.e. DPA at baseline and DXA at follow-up)<sup>(74)</sup>. However, DXA  
260 scans have been shown to produce lower results than DPA scans<sup>(75)</sup>, hence the effect size in this study  
261 may be more modest than the true result.

262 A potential role for vitamin C in fracture prevention has only been investigated in one previous  
263 prospective study of 918 US men and women with a mean age of 75 years. There was a risk reduction  
264 in hip fracture of 44% for supplemental vitamin C intake (mean: 260 mg/d compared to 0 mg/d) and  
265 of 69% for total (dietary and supplemental) vitamin C intake (mean: 313 mg/d compared to 94 mg/d)  
266 after 15-17 years of follow-up (RR and 95% CI not reported), although no significant risk reductions  
267 were found at other fracture sites<sup>(76)</sup>. As this study was comparatively small, further large prospective  
268 cohort studies of older men and women with long follow-up, which investigate fractures as the  
269 clinical endpoint of osteoporosis, are needed.

270 In summary, there are only limited data from three prospective and longitudinal studies  
271 investigating potential associations between vitamin C and bone health. Although these prospective  
272 studies meet the BHC of temporality, it is difficult to assess the strength of the associations and the  
273 potential for a dose-response relationship as not all studies reported effect sizes. Moreover, issues  
274 regarding analogy, inferring the absence of another confounder related to the predictor variable, and  
275 consistency were present. A greater number of prospective and longitudinal studies and more  
276 concordant adjustment for confounding factors may help establish more consistent findings of the  
277 relationship between vitamin C intake and osteoporosis and associated fractures. Moreover, the lack  
278 of evidence for a relation between vitamin C status and bone health needs to be investigated further  
279 as the only study investigating this did not adjust for age, gender and smoking<sup>(73)</sup>.

280

281 ***Case-control studies***

282 Case-control studies, summarised in Table 3, are used to examine specific exposures as potential risk  
283 factors of a disease in people with and without the condition. Recall bias, where **case subjects** tend to  
284 have a better recollection of specific exposures than the controls, and selection bias, resulting from  
285 both outcomes being pre-defined, are common issues of these studies. To date, three case-control  
286 studies have consistently shown that osteoporosis and fracture patients had lower serum vitamin C  
287 concentrations (cases: 17-37  $\mu\text{mol/L}$ ; controls: 23-54  $\mu\text{mol/L}$ ) and lower plasma vitamin C  
288 concentrations (cases: 30  $\mu\text{mol/L}$ ; controls: 55  $\mu\text{mol/L}$ ) than controls<sup>(77-79)</sup>. Only one study reported  
289 differently, but the authors inferred that their findings reflected most recent changes in food intake<sup>(80)</sup>.

290 In contrast to vitamin C status measures, findings for potential differences in dietary vitamin  
291 C intakes between cases and controls are less consistent<sup>(79,80)</sup>. Differences in measures of dietary  
292 intake and relatively small sample sizes may explain some of these inconsistent findings. However,  
293 associations with osteoporosis and fracture risk were reported when population intakes were stratified  
294 into quartiles of dietary vitamin C intake. For example, one case-control study showed a marginally  
295 significant fracture risk reduction for participants in the second quartile of vitamin C intake compared  
296 to the first (OR 0.39, 95% CI 0.15, 1.00; vitamin C intake range: 204-247 mg/d compared to  $\leq 203$   
297 mg/d)<sup>(79)</sup>. This was not significant for higher vitamin C intakes, possibly due to the high vitamin C  
298 intake of the study population (mean: 200 mg/d). Moreover, another case-control study reported that  
299 those in the third quartile of vitamin C intake had a significantly reduced risk of osteoporosis referent  
300 to the lowest quartile (OR 0.29, 95% CI 0.09, 0.96; vitamin C intake range: 137-176 mg/d compared  
301 to  $\leq 92$  mg/d)<sup>(81)</sup>. Recall bias in this study was low due to the diagnosis of osteoporosis at screening  
302 and the subsequent reporting of current vitamin C intake.

303 In conclusion, published case-control studies of osteoporosis and fracture patients have  
304 reported consistently lower blood vitamin C concentrations but not dietary intake of vitamin C. Thus,  
305 the BHC of consistency is currently not fulfilled. Although reported effect sizes appear to be large,  
306 this evidence is currently limited to only two studies. More case-control studies are needed to help  
307 clarify the discrepancies in vitamin C intake between osteoporosis and fracture patients and matched  
308 controls currently reported in the literature.

309

### 310 *Cross-sectional studies*

311 Cross-sectional studies are used to report the prevalence of a disease in a defined population at a  
312 specific point in time. Whether the exposure predated the disease or not cannot be determined.  
313 Previous cross-sectional studies are summarised in Table 4. Positive associations indicated that higher  
314 dietary vitamin C intake was associated with 3-5% higher BMD<sup>(6)</sup> and every 100 mg/d increment in  
315 vitamin C intake was associated with 0.01-0.02  $\text{g/cm}^2$  higher BMD<sup>(11,12)</sup>, although there is currently  
316 limited understanding of this clinical relevance. Moreover, users of vitamin C supplements (mean

317 [range] = 745 mg/d [70-5000 mg/d]) had 4% higher BMD and users of supplement doses of  $\geq 1000$   
318 mg/d had 14% higher BMD than non-users<sup>(82)</sup>. Although positive associations between dietary  
319 vitamin C intake and supplements and bone density have previously been reported, findings have  
320 been inconsistent<sup>(8,9,74,83-85)</sup>. The use of different dietary assessment methods as means of measuring  
321 vitamin C intake and differences in the adjustment for confounding factors may explain some of these  
322 discrepancies. Dietary methods have included semiquantitative FFQs with 97–126 food  
323 items<sup>(6,8,11,74,84,86,87)</sup>, three to seven-day food diaries<sup>(9,88)</sup> and 24-hour recalls<sup>(12,83)</sup>. Moreover, total  
324 (dietary and supplemental) vitamin C intake has not been linked with BMD in women<sup>(83,84,88)</sup>; and  
325 both positive and negative associations have been reported in men<sup>(74)</sup>, although the latter findings  
326 may have been biased by the population's smoking behaviour. Dietary intakes of vitamin C have  
327 previously been shown to be significantly lower in smokers than non-smokers<sup>(65)</sup> and serum vitamin  
328 C levels are lower in smokers independent of dietary intakes<sup>(64,65)</sup>. Hence, the exclusion of smokers  
329 to the study may have led to more consistent findings.

330 Potential associations between vitamin C from the diet or in serum and fracture risk have  
331 currently been examined in only one cross-sectional study of more than 13000 men and women aged  
332 20-90 years<sup>(12)</sup>. Findings were non-significant, although men with mean dietary vitamin C intakes of  
333 200 mg/d reported fewer fractures than men with higher or lower intakes. One may be critical about  
334 the large age range of the study population. As osteoporosis and associated fractures are known to be  
335 more prevalent in the elderly population<sup>(4)</sup>, the inclusion of very young participants may be an  
336 explanation for the non-significant findings.

337 Cross-sectional data on vitamin C and markers of bone homeostasis are sparse with only two  
338 studies investigating potential associations. One study found that higher intakes of vitamin C were  
339 associated with lower excretion of deoxypyridinoline (*no effect size shown*), indicating reduced bone  
340 resorption<sup>(8)</sup>. Similarly, the other study reported a significant association between the duration of  
341 vitamin C supplement use and markers of bone resorption, with serum CTX concentrations being  
342 0.022 pg/mL lower for every 1-year supplement use increment<sup>(85)</sup>.

343 Although there are data from a number of cross-sectional studies investigating vitamin C and  
344 bone health associations, the BHC of consistency, analogy and temporality were not fulfilled. The  
345 effect sizes of present cross-sectional studies are comparable to those previously reported for other  
346 dietary factors including potassium, although many studies did not report effect sizes. The limited  
347 number of Bradford Hill criteria currently fulfilled by cross-sectional studies may indicate that the  
348 reported associations between vitamin C intake and osteoporosis and fractures are less reliable  
349 evidence than relationships reported by prospective cohort studies and RCTs.

350

351 In summary, support for **studies, which have investigated the potential underlying mechanisms**  
352 **between vitamin C and osteoporosis prevention**, has come from a variety of epidemiological studies,  
353 although differences in study populations, dietary exposure, outcome measures and use of  
354 confounding factors in statistical analyses may have resulted in inconsistent findings. Current  
355 observational data are particularly limited for men as most studies have consisted of only women and  
356 for biological markers of vitamin C status which may be less subjective to recall bias and factors  
357 influencing the vitamin C content of food<sup>(89)</sup>. More observational studies in the general population  
358 are needed to address these limitations.

359

### 360 **Moderate versus high vitamin C intakes**

361 Results from three observational studies have indicated that significant associations with bone health  
362 were surprisingly stronger for moderate rather than higher vitamin C intakes<sup>(6,79,81)</sup>. For example,  
363 vitamin C intake was significantly associated with higher bone density or a reduction in fracture risk  
364 for the second quartile<sup>(79)</sup> or for the third quartile<sup>(6,81)</sup> of vitamin C intake rather than the highest intake  
365 levels. Similar cross-sectional observations have been reported for associations with serum vitamin  
366 C levels<sup>(12)</sup>. This may suggest that vitamin C may be related to bone density in a bell-shaped dose-  
367 response fashion with intakes below and above the optimum not being beneficial. The potential  
368 underlying mechanisms for this may relate to the properties of vitamin C rather than bone tissue itself.  
369 It has previously been suggested that vitamin C may not only have antioxidant properties, but may  
370 also exhibit pro-oxidant traits at higher concentrations, as supplementation of men and women with  
371 500 mg of vitamin C per day was shown to promote oxidative DNA damage<sup>(90)</sup> which may also be  
372 relevant to osteoporosis. Moreover, there is evidence from *in vitro* studies of a vitamin C dose-  
373 dependent suppression of bone cell growth and differentiation as well as collagen type I  
374 synthesis<sup>(21,24,91)</sup>. For example, vitamin C concentrations of 50 µg/ml were optimal for stimulation of  
375 human osteoblast-like cell lines and collagen type I synthesis, whereas higher levels resulted in the  
376 inhibition of cell differentiation<sup>(21)</sup>. Another experimental study investigating bovine osteoblast-like  
377 cell proliferation observed similar effects, although vitamin C concentrations of 200 µg/ml were  
378 found to be most effective<sup>(24)</sup>. As suggested by the authors, the use of different cell types may be an  
379 explanation for the inconsistencies in optimal vitamin C concentrations in these cell culture studies.  
380 The potential bell-shaped dose-response relationship between vitamin C and indicators of bone health  
381 may also further explain the lack of positive results reported in the intervention studies discussed  
382 above which included high supplement doses of 500–1000 mg/d. The potentially detrimental effects  
383 of higher vitamin C concentrations on the skeleton need to be investigated further; and this is a crucial  
384 step towards establishing optimal vitamin C intake levels.

385

## 386 **Discussion and conclusions**

387 Evaluating the current evidence for a potential role of vitamin C in osteoporosis and fracture  
388 prevention according to the Bradford Hill criteria (BHC) in the absence of RCTs provides some  
389 clarity regarding causality<sup>(19)</sup>. The BHC of specificity, inferring that a cause leads to a single effect,  
390 cannot be met as biological functions of vitamin C are versatile. However, there is emerging  
391 experimental evidence for a potential role of vitamin C in bone health, thus fulfilling the BHC of  
392 biological plausibility. The mechanisms include the involvement of vitamin C in osteoclastogenesis  
393 via RANKL expression, osteoblastogenesis via PPAR- $\gamma$  expression<sup>(22,23)</sup> and collagen synthesis via  
394 stimulation of pro-collagen mRNA expression and the hydroxylation of collagen fibres<sup>(38-40)</sup>. A  
395 number of observational studies support these findings, thus the BHC of coherence between  
396 laboratory and epidemiological studies is met. However, differences in study populations, different  
397 methods of measuring dietary exposure, outcome measures and use of confounding factors in these  
398 observational studies may have resulted in inconsistent findings. Consequently, the BHC of  
399 consistency and analogy are currently not fulfilled. Addressing these limitations in future  
400 epidemiological studies may help establish more consistent results.

401 Most observational studies published to date were of a cross-sectional nature. Thus, the BHC  
402 of temporality, inferring that the exposure preceded the disease outcome, was not met, and more  
403 cohort studies in the general population are needed to overcome this problem. Moreover, evaluating  
404 the BHC of the strength of the association based on the evidence currently available in the literature  
405 leads to equivocal conclusions as a large number of studies did not report effect sizes of their findings.  
406 Future studies should report effect sizes to help understand the overall clinical relevance of vitamin  
407 C for the prevention of osteoporosis and fractures.

408 The present review has highlighted that potential associations between vitamin C and bone  
409 health may not follow an expected dose-response curve due the vitamin exhibiting antioxidant  
410 properties at lower and pro-oxidant traits at higher concentrations. Potentially detrimental effects on  
411 the skeleton from higher vitamin C concentrations need to be investigated further, as this may be an  
412 issue with vitamin C supplementation, and understanding this is a crucial step towards establishing  
413 optimal vitamin C intake levels for the general population.

414 The final BHC of evidence from intervention studies is currently not fulfilled; **although the**  
415 **conventional hierarchy of the validity of study designs may be less applicable to nutritional research,**  
416 **as cross-sectional studies tend to capture long-term dietary intake more so than intervention studies.**  
417 **Nevertheless,** published intervention studies were not designed to evaluate the independent effects of  
418 vitamin C supplementation on potential improvements in bone health as interventions included  
419 additional supplementation with vitamin E and exercise programmes. Overall, the data are limited as  
420 only one double-blind RCT and two intervention studies have investigated this and dietary intake was

421 not controlled for. Moreover, further issues regarding study design, inclusion and exclusion criteria,  
422 duration of treatment and sample size were present. To our knowledge, published RCTs investigating  
423 the potential link between vitamin C and bone that use a supplement containing vitamin C only are  
424 still lacking and are urgently needed.

425

426 In conclusion, over the last few decades, *in vitro* and *in vivo* studies have provided insights and  
427 knowledge as to how vitamin C may influence the mechanisms that benefit the skeleton; and  
428 observational studies have provided some evidence for a potential role of vitamin C in osteoporosis  
429 and fracture prevention. However, data are limited as good quality studies are scarce and more  
430 investigations, particularly well-designed RCTs, are urgently needed to address the limitations  
431 outlined in this review.

432

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438

### 439 **Conflicts of interest**

440 There are no conflicts of interest.

441

### 442 **Authorship**

443 A. A. W. was the principal investigator and initiated the study. H. F. conducted the literature review  
444 and drafted the manuscript. A. A. W. and A. R. H. directed the preparation of the manuscript and A.  
445 A. W., A. R. H and A. J. contributed significantly to the drafting and critical reviewing of the article.  
446 All authors read and approved the final manuscript.

447

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**Table 1.** Summary of intervention studies investigating the effects of vitamin C on bone mineral density and markers of bone turnover.

Study	Subjects	Duration; study design	Age (yrs)	Primary outcome	Intervention	Results*	Comments
Maimoun <sup>(71)</sup> 2008 France	n 13 (4 men, 9 women)	2 months; /	69 - 79	BSALP, OC and CTX	No groups. All participants received the following treatment: 60 min of aerobic exercise 3 times/wk, <b>vit. C</b> (500 mg/d) & <b>vit. E</b> (100 mg/d)	M	BSALP concentration decreased <b>sig.</b> by 14.5% ( <i>P=Data not reported</i> ).
Chuin <sup>(72)</sup> 2009 Canada/France	n 34 (women)	6 months; randomised, controlled pilot study	61 - 73	FN and LS BMD	4 groups. Placebo group ( <i>n</i> 7): placebo (lactose); <b>Vit.</b> group ( <i>n</i> 8): ascorbic acid (1,000 mg/d) & $\alpha$ -tocopherol (600 mg/d); Exercise & placebo group ( <i>n</i> 11): 60 min of resistance training 3 times/wk & placebo (lactose); Exercise & <b>vit.</b> group ( <i>n</i> 8): 60 min of resistance training 3 times/wk & ascorbic acid (1,000 mg/d) & $\alpha$ -tocopherol (600 mg/d)	M	LS BMD decreased <b>sig.</b> by 1% in the placebo group (BMD pre: 1.01 $\pm$ 0.17 g/cm <sup>2</sup> ; BMD post: 1.00 $\pm$ 0.16 g/cm <sup>2</sup> ; <i>P</i> <0.05) but remained stable in the three intervention groups.
Ruiz-Ramos <sup>(70)</sup> 2010 Mexico	n 90 (25 men, 65 women)	12 months; double-blind RCT	68	TH and LS BMD	3 groups: Placebo group ( <i>n</i> 30): placebo ( <i>no details</i> ); Low <b>vit.</b> group ( <i>n</i> 30): ascorbic acid (500 mg/d) & $\alpha$ -tocopherol (400 IU/d); High <b>vit.</b> group ( <i>n</i> 30): ascorbic acid (1000 mg/d) & $\alpha$ -tocopherol (400 IU/d)	M	The high <b>vit.</b> group lost <b>sig.</b> less <b>TH</b> bone compared to the placebo group ( <i>Details not reported</i> ).

BSALP, alkaline phosphatase; OC, osteocalcin; CTX, collagen type 1 cross-linked C-telopeptide; **vit.**, vitamin; **sig.**, significant(ly); FN, femoral neck; LS, lumbar spine; BMD, bone mineral density; RCT, randomised placebo-controlled trial; TH, total hip.

\* Results were significant (S), non-significant (NS) or of mixed nature (M).



**Table 2.** Prospective and longitudinal studies assessing associations between vitamin C intake or status and bone mineral density or fracture risk.

Study	Follow-up	Subjects	Age (yrs)	Dietary assessment	Vit. C intake (mg/d)*	Outcome measures and analyses	Results†	Comments
Kaptoge <sup>(73)</sup> 2003 UK	2-5 yrs	n 944 (470 men; 474 women)	72 (67-79)	7dD	Median (range) dietary intake: Tertile 1 = 73 (7-57) Tertile 2 = 78 (58-98) Tertile 3 = 132 (99-363) <i>Data for plasma levels not shown.</i>	2-5 year change in TH BMD stratified by tertiles of either dietary vit. C intake or plasma vit. C levels	Diet: M Plasma: NS	Women in tertile 2 and 3 of dietary vit. C intake had approx. 52% and 54% less TH BMD loss, respectively ( $P=0.015$ and $P=0.010$ ; $P$ -trend=0.016).
Sahni <sup>(74)</sup> 2008 US	4 yrs	n 606 (213 men; 393 women)	75	FFQ	Mean (SD) dietary intake: Men = 141 (73) Women = 158 (83) Mean (SD) suppl. intake: Men = 82 (235) Women = 95 (248) Group 1 = 0 Group 2 < 90 / 75‡ Group 3 ≥ 90 / 75‡ Mean (SD) total intake: Men = 223 (259) Women = 253 (267) <i>Intake data for tertiles not shown.</i>	4-year change in LS, FN, T and RS BMD stratified by tertiles of dietary or total vit. C intake or categories of suppl. vit. C intake and either calcium intake, vit. E intake, smoking or oestrogen use	Diet: M Suppl.: NS	LS and T BMD loss was sig. less with higher dietary vit. C intakes in men ( $P$ -trend≤0.05). FN and T BMD loss was sig. less for higher total vit. C intake among men with low calcium intakes and with low total vit. E intakes ( $P$ -trend≤0.03). A 102% reduction in T BMD loss between extreme tertiles of total vit. C intake among men with low calcium intakes ( $P<0.05$ ).
Sahni <sup>(76)</sup> 2009 US	15-17 yrs	n 918 (39.1% men; 60.9% women)	75	FFQ	Median (range) dietary intake: Tertile 1 = 86 Tertile 2 = 133 Tertile 3 = 208 Suppl. intake: Tertile 1 = 0 Tertile 2 < 75 Tertile 3 ≥ 75 Median (range) total intake: Tertile 1 = 94 / 95§ Tertile 2 = <i>Data not shown</i> Tertile 3 = 313 / 308§	Risk of hip fracture or non-vertebral fracture stratified by tertiles of dietary, suppl. or total vit. C intake in the combined sample of men and women	Diet: NS Suppl.: M Total: M	A reduction in hip fracture of 69% between extreme tertiles of suppl. vit. C intake ( $P=0.007$ ; $P$ -trend=0.02) and of 44% for total vit. C intake ( $P=0.04$ ; $P$ -trend=0.04).

Vit., vitamin; 7dD, 7-day food diary; TH, total hip; BMD, bone mineral density; approx., approximately; FFQ, food frequency questionnaire; suppl., supplement(al); LS, lumbar spine; FN, femoral neck; T, trochanter; RS, radial shaft; sig., significant(ly).

\* Total intake is the sum of dietary intake and intake from supplements.

† Results were significant (S), non-significant (NS) or of mixed nature (M).

‡ Data shown for men / women.

§ Data shown for hip / non-vertebral fracture analyses.

**Table 3.** Case-control studies assessing vitamin C intake or status in osteoporosis and fracture patients in comparison to controls.

Study	Subjects	Age (yrs)	Dietary assessment	Mean or range <b>vit. C</b> intake or blood <b>conc.</b>	Outcome measure(s) and analyses	Results*	Comments
Falch <sup>(77)</sup> 1998 Norway	<i>n</i> 40 hip fracture cases; 102 controls (men and women)	83	N/A	Serum <b>conc.</b> : CA = 37 µmol/L, CO = 50 µmol/L Serum <b>conc.</b> in 20 age-matched case-control pairs: CA = 34 µmol/L, CO = 54 µmol/L	Serum <b>vit. C conc.</b> in cases and controls or in 20 case-control pairs matched for age	Serum: S	Serum <b>vit. C conc.</b> were significantly lower in cases than in controls ( <i>P</i> <0.01).
Lumbers <sup>(80)</sup> 2001 UK	<i>n</i> 75 hip fracture cases; 50 controls (women)	80 (61-103)	three 24hR	Dietary intake: CA = 60.7 mg/d, CO = 55.2 mg/d Plasma <b>conc.</b> : CA = 42.7 µmol/L, CO = 20.8 µmol/L	<b>Vit. C</b> intakes or plasma <b>conc.</b> in cases and controls	Intake: NS Plasma: S	Plasma <b>conc.</b> were significantly higher in cases than in controls ( <i>P</i> <0.001).
Maggio <sup>(78)</sup> 2003 Italy	<i>n</i> 75 osteoporosis cases; 75 controls (women)	60+	N/A	Plasma <b>conc.</b> : CA = 30.0 µmol/L, CO = 55.5 µmol/L	Plasma <b>vit. C conc.</b> in cases and controls	Plasma: S	Cases had <b>sig.</b> lower plasma <b>vit. C conc.</b> than controls ( <i>P</i> <0.001).
Martinez-Ramirez <sup>(79)</sup> 2007 Spain	<i>n</i> 167 fracture cases; 167 controls (20% men; 80% women)	65+	FFQ	Intake: CA = 268 mg/d, CO = 275 mg/d Quartile 1 ≤ 203 mg/d Quartile 2 = 204-247 mg/d Quartile 3 = 248-334 mg/d Quartile 4 > 334 mg/d Serum <b>conc.</b> : CA = 17.6 µmol/L, CO = 23.3 µmol/L Quartile 1 ≤ 8.4 µmol/L Quartile 2 = 8.5-19.6 µmol/L Quartile 3 = 19.7-34.1 µmol/L Quartile 4 > 34.1 µmol/L	<b>Vit. C</b> intakes or serum <b>conc.</b> in cases and controls and in association with fracture risk	Intake: M Serum: S	A marginal <b>sig.</b> fracture risk reduction for quartile 2 <b>versus</b> 1 of <b>vit. C</b> intake (OR 0.39, 95% CI 0.15, 1.00; <i>P</i> -trend=0.87). Mean serum <b>conc.</b> were <b>sig.</b> lower in cases than in controls ( <i>P</i> =0.012). A <b>sig.</b> reduction in fracture risk for quartile 4 <b>versus</b> 1 of serum <b>conc.</b> (OR 0.31, 95% CI 0.11, 0.87; <i>P</i> -trend=0.03).
Park <sup>(81)</sup> 2011 South Korea	<i>n</i> 72 osteoporosis cases; 72 controls (women)	50-70	FFQ	Dietary intake: Quartile 1 ≤ 91.5 mg/d Quartile 2 = 91.5-136.9 mg/d Quartile 3 = 136.9-176.3 mg/d Quartile 4 > 176.3 mg/d	Dietary <b>vit. C</b> intake & risk of osteoporosis	Intake: S	A <b>sig.</b> reduction in the risk of osteoporosis for quartile 3 <b>versus</b> 1 of dietary <b>vit. C</b> intake (OR 0.29, 95% CI 0.09, 0.96; <i>P</i> -trend=0.24).

*Vit., vitamin; conc., concentration(s); N/A, not applicable; CA, cases; CO, controls; 24hR, 24-hour dietary recall; FFQ, food frequency questionnaire.*

*\*Results were significant (S), non-significant (NS) or of mixed nature (M).*

**Table 4.** Cross-sectional studies assessing associations between vitamin C intake or status and bone mineral density, markers of bone turnover or fracture risk.

Study	Subjects	Age (yrs)	Dietary assessment	Mean (SD); range vit. C intake* or blood conc.	Outcome measures and analyses	Results†	Comments
Sowers <sup>(83)</sup> 1985 US	n 324 (women)	67 (55-80)	24hR	Total intake: Low calcium group = 211 (351) mg/d Low calcium group = 268 (309) mg/d	Association between MR BMD and vit. C intake	Total: NS	Vit. C intake was only marginally associated with MR BMD ( <i>Effect size not shown</i> ; $P=0.051$ ).
Leveille <sup>(86)</sup> 1997 US	n 1892 (women)	72 (55-64)	FFQ	Dietary intake = 113 (52); 12-399 mg/d Suppl. intake = 294 (447); 0-2500 mg/d Duration of suppl. use: Group 1 = non-user Group 2 = 1-5 yrs Group 3 = 5-10 yrs Group 4 $\geq$ 10 yrs Total intake = 407 (454); 13-2560 mg/d	FN BMD stratified by vit. C intake or FN BMD stratified by duration of vit. C suppl. use and either age groups (55-64yrs, 65-74yrs and 75+) or oestrogen use	Diet: NS Suppl: M Total: NS	Approx. 6.7% and 3.2% higher FN BMD for longest supplement users compared to non-users in women aged 55-64yrs ( $P=0.02$ ; $P$ -trend=0.01) and in women who had never taken oestrogen ( $P=0.02$ ; $P$ -trend=0.02), respectively.
New <sup>(6)</sup> 1997 UK	n 994 (women)	47 (44-50)	FFQ	Dietary intake = 126 (96); 16-1164 mg/d <i>Intake data for quartiles not shown.</i>	LS, FN, T and WT BMD stratified by quartiles of dietary vit. C intake	Diet: S	Dietary vit. C intake correlated sig. with LS BMD ( $r^2$ 0.10; $P<0.001$ ). Approx. 4.5% higher LS BMD ( $P<0.002$ ), 3% higher FN BMD ( $P<0.01$ ) and higher T and WT BMD ( <i>Effect sizes not shown</i> ; $P<0.02$ ) for quartile 3 <i>versus</i> 1 of dietary vit. C intake.
Hall <sup>(11)</sup> 1998 US	n 775 (women)	56 (45-64)	FFQ	Dietary intake = 140 (76) mg/d <i>Note: dietary calcium intake: Low (n 199) &lt; 500 mg/d High (n 574) &gt; 500 mg/d</i>	LS, FN and TH BMD stratified by 100mg/d increments of dietary vit. C intake with and without additional stratification by low and high dietary calcium intake	Diet: M	FN and TH BMD were 0.017 g/cm <sup>2</sup> higher for each 100 mg/d increase in dietary vit. C intake ( $P=0.002$ and $P=0.005$ ). For every 100 mg/d increment in dietary vit. C intake, LS, FN and TH BMD increased sig. by 0.0199 g/cm <sup>2</sup> ( $P=0.024$ ), 0.0190 g/cm <sup>2</sup> ( $P=0.002$ ) and 0.0172 g/cm <sup>2</sup> ( $P=0.010$ ), respectively, in those with high calcium intakes.
New <sup>(8)</sup> 2000 UK	n 62 (women)	47 (45-54)	FFQ	Dietary intake = 103 (66); 24-453 mg/d <i>Intake data for quartiles not shown.</i>	LS, FN, T, WT and forearm BMD and PYD, DPD and OC stratified by quartiles of dietary vit. C intake	Diet: M	Sig. lower mean DPD excretion across quartiles of dietary vit. C intake ( <i>Effect size not shown</i> ; $P$ -trend<0.02).
Morton <sup>(82)</sup> 2001 US	n 994 (women)	72 (50-98)	N/A	Suppl. intake: Non-users = 0 mg/d Users = 745 mg/d; 70-5000 mg/d Group 1 = 0 mg/d (non-users) Group 2 $\leq$ 500 mg/d Group 3 $\geq$ 1000 mg/d	LS, FN, TH, MR and UR BMD stratified by use of vit. C suppl. with and without additional stratification by	Suppl.: M	4.1% higher FN BMD for suppl. users compared to non-users ( $P=0.02$ ). For current users of oestrogen, calcium and vit. C suppl., BMD was higher by approx. 6% at the TH ( $P=0.05$ ), 9% at the FN ( $P=0.0001$ ) and 12% at the UR ( $P=0.02$ ) compared to non-vit. C users. Approx. 14%

					oestrogen use or by oestrogen and calcium use; and BMD stratified by dose of <b>vit. C</b> suppl.		higher UR BMD for women with the highest <b>vit. C</b> suppl. dose compared to non-users ( $P<0.05$ ; $P$ -trend $<0.04$ ).
Simon <sup>(12)</sup> 2001 US	<i>n</i> 13080 (6137 men; 6943 women); ( <i>n</i> 11849 for BMD analyses)	(20-90)	24hR	Men: Dietary intake = 102 (104) mg/d Serum <b>conc.</b> = 38.0 (23.8) $\mu\text{mol/L}$ Pre-menopausal women: Dietary intake = 81 (83) mg/d Serum <b>conc.</b> = 43.7 (25.6) $\mu\text{mol/L}$ Post-menopausal women: Dietary intake = 88 (80) mg/d Serum <b>conc.</b> = 50.5 (27.8) $\mu\text{mol/L}$	TH BMD or self-reported fractures stratified by 100 mg/d increments in dietary <b>vit. C</b> intake or by SD increments in serum ascorbic acid <b>conc.</b>	Diet: M Serum: M	In men, TH BMD was highest at serum ascorbic acid <b>conc.</b> between about 28.4-56.8 $\mu\text{mol/L}$ and self-reported fractures were least common at dietary <b>vit. C</b> intakes of about 200 mg/d; whereas higher and lower <b>conc.</b> were associated with lower TH BMD ( $P<0.05$ ) and a higher self-reported fracture prevalence ( $P=0.01$ ). In pre-menopausal women, TH BMD was 0.01 $\text{g/cm}^2$ higher for every 100 mg/d increase in dietary <b>vit. C</b> intake ( $P=0.002$ ).
Hich <sup>(88)</sup> 2003 US	<i>n</i> 136 (women)	69 (57-88)	3dD	Dietary intake = 128 (70); 23-402 mg/d	Dietary <b>vit. C</b> intake as a predictor of WB BMD and BMC and of TH, FN, WT, T, RS, UR and hand BMD	Diet: S	Dietary <b>vit. C</b> intake was a predictor of BMD of more than 1% for TH ( $P=0.012$ ), T ( $P=0.047$ ) and RS ( $P=0.027$ ) BMD and a marginally <b>sig.</b> predictor of WT BMD ( $P=0.052$ ).
Wolf <sup>(84)</sup> 2005 US	<i>n</i> 11068 (women)	63 (50-79)	FFQ	Dietary intake = 84 (49) mg/d Total intake = 170 (182) mg/d	WB, LS, TH, FN and T BMD stratified by dietary or total <b>vit. C</b> intake with or without additional stratification by either calcium intake, smoking or HRT use	Diet: NS Total: NS	A <b>sig.</b> positive interaction effect between HRT use and total <b>vit. C</b> intake for WB ( $P=0.045$ ), LS ( $P=0.03$ ), TH ( $P=0.029$ ) and FN ( $P=0.004$ ) BMD.
Pasco <sup>(85)</sup> 2006 Australia	<i>n</i> 533 (women)	56-82	N/A	Duration of <b>suppl.</b> use ( <b>vit. C</b> + E): Group 1 = 0 yrs (non-user) Group 2 < 5 yrs Group 3 $\geq$ 5 yrs	WB BMD, serum CTX and BSALP stratified by use or duration of <b>vit. C</b> and E <b>suppl.</b>	Suppl.: M	The duration of <b>vit. C</b> and E <b>suppl. use</b> ( $\geq 5$ years) was associated with <b>sig.</b> lower CTX <b>conc.</b> compared to non- <b>suppl.</b> users ( $P<0.05$ ). CTX <b>conc.</b> were 0.022 pg/mL lower for each year of <b>vit. suppl.</b> use ( $P=0.05$ ).
Prynne <sup>(9)</sup> 2006 UK	<i>n</i> 257 (111 boys; 101 girls); <i>n</i> 67 (older women)	17 (16-18); 68 (60-83)	7dD	Dietary intake: Boys = 96 mg/d Girls = 95 mg/d Older women = <i>Data not shown.</i>	WB, LS, TH, FN and T BMD stratified by <b>vit. C</b> intake	Diet: M	In boys, each 100% change in <b>vit. C</b> intake was associated with a 3-5% change in BMD at all sites ( $P<0.05$ ).

Sahni <sup>(74)</sup> 2008 US	n 874 (334 men; 540 women)	75	FFQ	Dietary intake: Men = 141 (73) mg/d Women = 158 (83) mg/d Suppl. intake: Men = 82 (235) mg/d Women = 95 (248) mg/d Group 1 = 0 mg/d Group 2 < 90 / 75 mg/d‡ Group 3 ≥ 90 / 75 mg/d‡ Total intake: Men = 223 (259) mg/d Women = 253 (267) mg/d <i>Intake data for tertiles not shown.</i>	LS, FN, T and RS BMD stratified by tertiles of dietary or total vit. C intake or categories of suppl. vit. C intake and either calcium intake, vit. E intake, smoking or oestrogen use	Diet: NS Suppl.: M Total: M	In men, total vit. C intake was positively associated with FN BMD among never-smokers ( <i>P</i> -trend=0.04). In current smokers, total and suppl. vit. C intake were negatively associated with T BMD ( <i>P</i> -trends=0.01).
Sugiura <sup>(87)</sup> 2011 Japan	n 293 (women)	60	FFQ	Dietary intake = 170 (161-179) mg/d§ Tertile 1 = 47-139 mg/d  Tertile 2 = 140-214 mg/d  Tertile 3 = 215-625 mg/d	Risk of low radial BMD stratified by tertiles of dietary vit. C intake	Diet: S	<b>Sig.</b> lower risk of low radial BMD for tertile 3 vs 1 of dietary vit. C intake (OR 0.25, 95% CI 0.07, 0.82; <i>P</i> -trend=0.01).

*Vit., vitamin; conc., concentration(s); 24hR, 24-hour dietary recall; MR, mid radius; BMD, bone mineral density; FFQ, food frequency questionnaire; suppl., supplement(al); FN, femoral neck; approx., approximately; LS, lumbar spine; T, trochanter; WT, Ward's triangle; sig., significant; TH, total hip; PYD, pyridinoline; DPD, deoxypyridinoline; OC, osteocalcin; N/A, not applicable; UR, ultradistal radius; 3dD, 3-day food diary; WB, whole body; RS, radial shaft; CTX, collagen type 1 cross-linked C-telopeptide; BSALP, bone-specific alkaline phosphatase; 7dD, 7-day food diary.*

\* Total intake is the sum of dietary intake and intake from supplements.

† Results were significant (S), non-significant (NS) or of mixed nature (M).

‡ Data shown for men / women.

§ Geometric mean (95% CI).

| Intake range.