University of East Anglia

SCIENTIFIC REPORT submitted to EFSA

Literature search and review related to specific preparatory work in the establishment of Dietary Reference Values

Preparation of an evidence report identifying health outcomes upon which Dietary Reference Values could potentially be based for magnesium, potassium and fluoride [1]

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[1] **(Question No EFSA-Q-2010-01547). Accepted for publication on 14 May 2012.**

Overall summary

The aim of these systematic searches and reviews was to collate all of the scientific data from which Dietary Reference Values for magnesium (Mg), potassium (K) and fluoride (F) may be derived, building on existing information in the Scientific Committee for Food Dietary Reference Values report of 1993.

In March, September and October 2011, electronic searches were run after rigorous development and optimisation of the complex search strategy (which included indexing and text terms, truncation and Boolean operators). Databases searched were Medline, EMBASE (both on Ovid SP) and the Cochrane Library CENTRAL. Additional references were identified by checking reference lists in key reviews, included studies and DRV reports.

Search results were imported into EndNote®, duplicates were removed and the library was then screened for studies that appeared to be relevant. Each full-text article retrieved was assessed using an inclusion/exclusion form. The literature search focused on: 1) data published in the English language from 1990 onwards; 2) human studies conducted in generally healthy populations; 3) studies which examined the relationship between micronutrient intake, status, and/or health; and 4) studies which were relevant to micronutrient intakes within the normal dietary/physiological range.

A total of 7887 articles (Mg n=3123; K n=2583; F n=2181) were screened on the basis of title and abstract, resulting in the selection of 848 articles (Mg n=359; K n=235; F n=254) for full-text assessment, and the final inclusion of 135 studies (Mg n=48; K n=44; F n=43) for this review. Data on the study design, methods and results were extracted and study quality was assessed. All stages in the process were duplicated by researchers at a level of 10% to ensure consistency in data recording.

Included studies reported on: micronutrient bioavailability; nutrient interactions; metabolism; status markers; breast milk micronutrient concentration; polymorphisms; and micronutrient specific health outcomes (cardiovascular risk factors; blood pressure; bone health; tooth health; aldosterone and renin; diabetes; metabolic rate; sleep; and leptin levels). The majority of studies identified were assessed as being at high risk of bias (n=119), with the remainder of studies at moderate (n=12) or low risk of bias (n=4). Data were generally more limited for children (with the exception of fluoride studies addressing tooth health). Overall, there appeared to be a lack of high quality studies on which to base Dietary Reference Values for magnesium, potassium and fluoride.

Key words: magnesium, potassium, fluoride, systematic review, Dietary Reference Values (DRVs), dietary requirements, dietary intake, health outcomes, biomarkers, status, bioavailability

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Background

In 2005, the European Food Safety Authority (EFSA) received a mandate from the European Commission to review the existing advice of the Scientific Committee for Food (SCF) published in 1993 on Dietary Reference Values (DRVs) for energy, macro- and micronutrients and other substances with a nutritional or physiological effect. Ten micronutrients were selected by the NDA Panel as a priority for further review of existing data, which were vitamins A, C, E, K (Lot 1), chromium, manganese, molybdenum (Lot 2), and magnesium, potassium, fluoride (Lot 3).

In order to review each micronutrient it was established that a comprehensive literature search should be carried out to identify health outcomes upon which DRVs could potentially be based for different life stages and gender groups of the general (healthy) population. Health outcomes comprise suitable indicators of nutrient adequacy used to define nutrient requirements, such as prevention of clinical deficiency symptoms, maintenance of nutrient-related functional competence, maintenance of cell (organ) integrity, achievement of sufficient nutrient body stores or status. In addition, if available, the scientific evidence for the relationship between dietary nutrient intake and risk of chronic disease, such as diabetes, cardiovascular disease or cancer, should also be considered.

It was agreed in the tender specification that the scientific literature should be searched comprehensively for data published from January 1990 onwards in order to update the current SCF recommendations (SCF, 1993). The literature search should focus on primary studies in humans reporting on the (dose-response) relationship between quantitative intakes of the nutrients within the physiological (dietary) intake range and health outcomes on which DRVs may be based.

Terms of reference

Literature search and review related to specific preparatory work in the establishment of Dietary Reference Values, open call for tender CT/EFSA/NDA/03.

Acknowledgements

This contract was awarded by EFSA to:

Contractor: Department of Nutrition, Norwich Medical School, University of East Anglia, Norwich Research Park, Norwich, NR4 7TJ.

Contract: Literature search and review related to specific preparatory work in the establishment of Dietary Reference Values. Lot 3 – Preparation of an evidence report

identifying health outcomes upon which Dietary Reference Values could potentially be based for magnesium, potassium and fluoride.

Contract number: CT/EFSA/NDA/03

Objectives

The purpose of this work was to collate the scientific data from which Dietary Reference Values for magnesium, potassium and fluoride may be derived, building on existing advice of the Scientific Committee for Food Dietary Reference Values report of 1993.

The review was based on a systematic search and review approach, and relevant publication outcomes have been tabulated.

Materials and Methods

The materials and methods are summarised here for all micronutrients. There were some minor variations in the methodological approach for each micronutrient, as detailed individually within each micronutrient section.

1.1. Search strategy

In March, September and October 2011, the electronic searches were run following rigorous development and optimisation of the complex search strategy (which included indexing and text terms, truncation and Boolean operators, **Appendix A**). Databases searched were Medline, EMBASE (both on Ovid SP) and the Cochrane Library CENTRAL. Additional references were identified by checking reference lists of key reviews, pertinent included studies and key DRV reports (IOM, 2004; 1997; NHMRC, 2006).

Search results were imported into EndNote® (version X4, Thomson Reuters, New York), duplicates were removed and the library was then screened on the basis of title and abstract for relevance to the review. Initially, 10% of the titles and abstracts were assessed by multiple reviewers in order to ensure there was consistency and agreement regarding the type of studies to include, the remaining 90% was then screened by a single reviewer. The initial screening excluded articles that were obviously irrelevant using a few key criteria: non-English language articles, articles not concerning the relevant micronutrient, IV as opposed to oral administration etc. Only original articles or systematic reviews were considered (non-systematic reviews, conference abstracts and letters were excluded). Where it was unclear if the study was suitable the full-text was collected and assessed.

1.2. Inclusion and screening full text assessment

Each full-text article retrieved was assessed for inclusion using an inclusion/ exclusion form. The exclusion criteria applied were:

- Articles published before 1990.
- Language other than English.
- Non-systematic reviews, conference abstracts, letters.
- Animal, cell lines, *in-vitro* trials.
- Irrelevant populations (elite athletes, or those with an illness likely to affect relevance to DRV setting).
- No relationship between i) intake and status; ii) intake and health; iii) status and health (with the exception of studies reporting relevant micronutrient concentrations in breast milk).
- Supplements not orally ingested.
- Supplements not approved by the EC (EC Directive 2002/46/EC; EC Regulation No 1925/2006).
- Data not relevant to doses below the UL (except when investigating balance and bioavailability factors).
- Studies with inappropriate methodology e.g. intervention studies not using a placebo treatment.

The full text assessment was completed with a minimum of 10% duplication. Where a decision could not be reached, the paper was discussed within the review group to reach a consensus on inclusion.

1.3. Data extraction

Information about included studies was extracted and tabulated in a standardised format into a Microsoft Excel® (Microsoft Corp, Seattle) spreadsheet, and 10% was checked by a second reviewer for completeness and accuracy of data extraction. Where there were multiple publications for a single study these were extracted as one, to avoid duplication of methodology. Data extracted included the following:

Study characteristics: including study reference, methodology, age, type and number of participants, country of origin, measures of intake or status and health outcome measures.

Study results: the numerical relationship(s) presented for the relevant intake-status-health associations (or other relevant data) presented in each paper (e.g. odds ratio or mean difference and confidence intervals).

Validity assessment: details of study quality and risk of bias for each study, assessed according to study methodology (see below).

1.4. Validity assessment

Study validity criteria were collated in the Microsoft Excel® (Microsoft Corp, Seattle) spreadsheet during the data extraction phase. The criteria were then assessed according to study methodology using a rigorous scheme devised during the work of the EURRECA Network of Excellence (Hooper *et al*, 2009). Studies were classified as high, moderate or low risk of bias according to each study methodology. Key criteria were:

SR: databases searched, inclusion criteria, validity assessment.

RCT: randomisation, allocation method, blinding, loss to follow up.

Cohort, case-control: similarity of groups at baseline, potential confounders (and their adjustment), intake assessment methods.

Cross-sectional: exposure and status assessment, potential confounders.

Case-study: number of cases explored and strength of measured change.

Balance: background exposure, measurement of dose provided.

Data extraction and validity assessment outcomes were analysed and reported as a narrative synthesis.

SCIENTIFIC REPORT submitted to EFSA

Literature search and review related to specific preparatory work in the establishment of Dietary Reference Values

Preparation of an evidence report identifying health outcomes upon which Dietary Reference Values could potentially be based for magnesium, potassium and fluoride [2]

Magnesium

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[2] **(Question No EFSA-Q-2010-01547). Accepted for publication on 14 May 2012.**

Abstract

The objective of this systematic search and review was to identify the scientific data from January 1990 to October 2011 upon which Dietary Reference Values (DRVs) may potentially be based for magnesium.

Articles were identified using Medline, EMBASE (both on Ovid SP) and the Cochrane Library CENTRAL databases. Duplicate references were removed and additional studies identified by checking reference lists of pertinent reviews, included studies and current reports on DRVs. A total of 3123 articles were screened on the basis of title and abstract, resulting in 359 articles retrieved for full-text assessment, and the final inclusion of 48 studies (49 articles) for this review.

The studies included reported on: cardiovascular risk factors (8); osteoporosis risk factors (2); magnesium metabolism (25); and breast milk magnesium concentration (13). Study designs included systematic review and meta-analysis, balance, isotope, depletion, randomised and non-randomised controlled trials and cross-sectional studies. The majority of studies included were assessed as being at high risk of bias (42), with the remainder of studies at moderate (4) and low risk of bias (2). Most studies were conducted in adult females, with only two studies conducted in children and two studies conducted exclusively in adult men. Overall high quality evidence from which DRVs for magnesium may be derived was limited.

Summary

This systematic search and review was carried out preparatory to work by EFSA to establish Dietary Reference Values (DRVs) for magnesium, potassium and fluoride, Lot 3 from the open call for tender CT/EFSA/NDA/03. This report summarises the findings on magnesium.

The literature was comprehensively searched from January 1990 to October 2011 for studies in the English language. The search focused on primary research in humans concerning maintenance of functional competence and the prevention of clinical deficiency and chronic disease upon which DRVs may be based. Only studies reporting a quantitative relationship between i) intake and status; ii) intake and health; or iii) status and health were included (with the exception of studies reporting magnesium concentration in breast milk).

Articles were identified using Medline, EMBASE (both on Ovid SP) and the Cochrane Library CENTRAL databases. Complex search strategies using index and text terms, truncating and Boolean operators were developed and refined for each database. The search results were combined and imported into Endnote® (version X4, Thomson Reuters, New York) duplicate references were removed, resulting in 3113 references to screen. A further ten articles were identified by checking reference lists of pertinent reviews, included studies and current reports on DRVs. In total, 3123 articles were screened on the basis of title and abstract, resulting in 359 articles being retrieved for full text assessment, and the final inclusion of 48 studies, reported in 49 articles, for this review which satisfied all inclusion

criteria. For each study, data on design, methodology, results and validity were fully extracted into a Microsoft Excel® (Microsoft Corp, Seattle) database, and the key data summarised.

The majority of studies included investigated magnesium metabolism and interactions with other nutrients (25). Other studies investigated cardiovascular risk factors (8), osteoporosis (2) and breast milk magnesium concentration (13). The studies were classified as: 1 SR; 1 meta-analysis of balance studies; 5 depletion; 13 balance; 2 balance/ isotope; 5 isotope; 7 RCTs; 1 CT; and 13 cross-sectional designs.

The effect of magnesium on cardiovascular and osteoporosis risk factors was fragmented and inconsistent. Similarly, dietary influences on magnesium metabolism were difficult to interpret. Evidence for status markers and magnesium balance was more comprehensive. The responsiveness of magnesium status markers was systematically reviewed by Witowski *et al* (2011), which included 27 individual studies. A significant response to magnesium intake was found for serum/ plasma, erythrocyte and urinary magnesium concentrations. However, the authors emphasise a paucity of high quality data in this area. Data for magnesium balance was pooled from 27 balance studies conducted at the US Department of Agriculture (Hunt and Johnson, 2006). Magnesium excretion increased linearly with intake, with zero balance predicted to occur at an intake of 165 mg magnesium/day. Data was reported for adults only.

Regarding data of relevance to DRV setting for infants, mean magnesium concentration in breast milk was found to vary from 23-47 mg/l.

The majority of included studies were assessed as being at high risk of bias (42), with the remainder at moderate (4) and low risk of bias (2). Overall, high quality data on which to derive DRVs for magnesium were limited.

Key words: magnesium, systematic review, Dietary Reference Values (DRVs), dietary requirements, health outcomes, biomarkers, status, bioavailability

Introduction

This report focused on identifying information to inform the setting of Dietary Reference Values for magnesium.

Magnesium is a cofactor for more than 300 enzymes, many of which are associated with energy metabolism. Magnesium is also involved in protein and nucleic acid synthesis; bone mineralisation; maintenance of Ca, K and Na homeostasis; and the maintenance of electrical potentials in nerve and muscle membranes (SCF, 2003; SCF, 2006). This review will focus on intakes below the Tolerable Upper Intake Level (UL), set at 250 mg/day (SCF, 2006). The UL was set for adults and children aged four years or over and did not include magnesium naturally present in foods and beverages.

This review only reports on magnesium forms present naturally in foods or those approved by the EC (EC Directive 2002/46/EC; EC Regulation No 1925/2006) for use in foods or food supplements:

Foods: magnesium acetate; magnesium carbonate; magnesium chloride; magnesium salts of citric acid; magnesium gluconate; magnesium glycerophosphate; magnesium salts of orthophosphoric acid; magnesium lactate; magnesium hydroxide; magnesium oxide; magnesium potassium citrate; magnesium sulphate.

Food supplements: magnesium acetate; magnesium L-ascorbate; magnesium bisglycinate; magnesium carbonate; magnesium chloride; magnesium salts of citric acid; magnesium gluconate; magnesium glycerophosphate; magnesium salts of orthophosphoric acid; magnesium lactate; magnesium L-lysinate; magnesium hydroxide; magnesium malate; magnesium oxide; magnesium L-pidolate; magnesium potassium citrate; magnesium pyruvate; magnesium succinate; magnesium sulphate; magnesium taurate; magnesium acetyl taurate.

Magnesium is widely distributed in both animals and plants. Key sources of magnesium are wholegrains, nuts, legumes, dark green vegetables, seafood, and water, with magnesium concentration being higher in 'hard' water (IOM, 1997; NHMRC, 2006; SCF, 2006).

The exchange of magnesium between body pools occurs slowly and biomarkers for magnesium are problematic. Indicators have included serum magnesium, plasma ionised magnesium, intracellular magnesium and magnesium balance or loading tests (Arnaud, 2008; IOM, 1997; NHMRC, 2006).

Specific objectives and methodology

The purpose of this work was to collate the scientific data from which Dietary Reference Values for magnesium may be derived, building on existing advice of the Scientific Committee for Food Dietary Reference Values report of 1993.

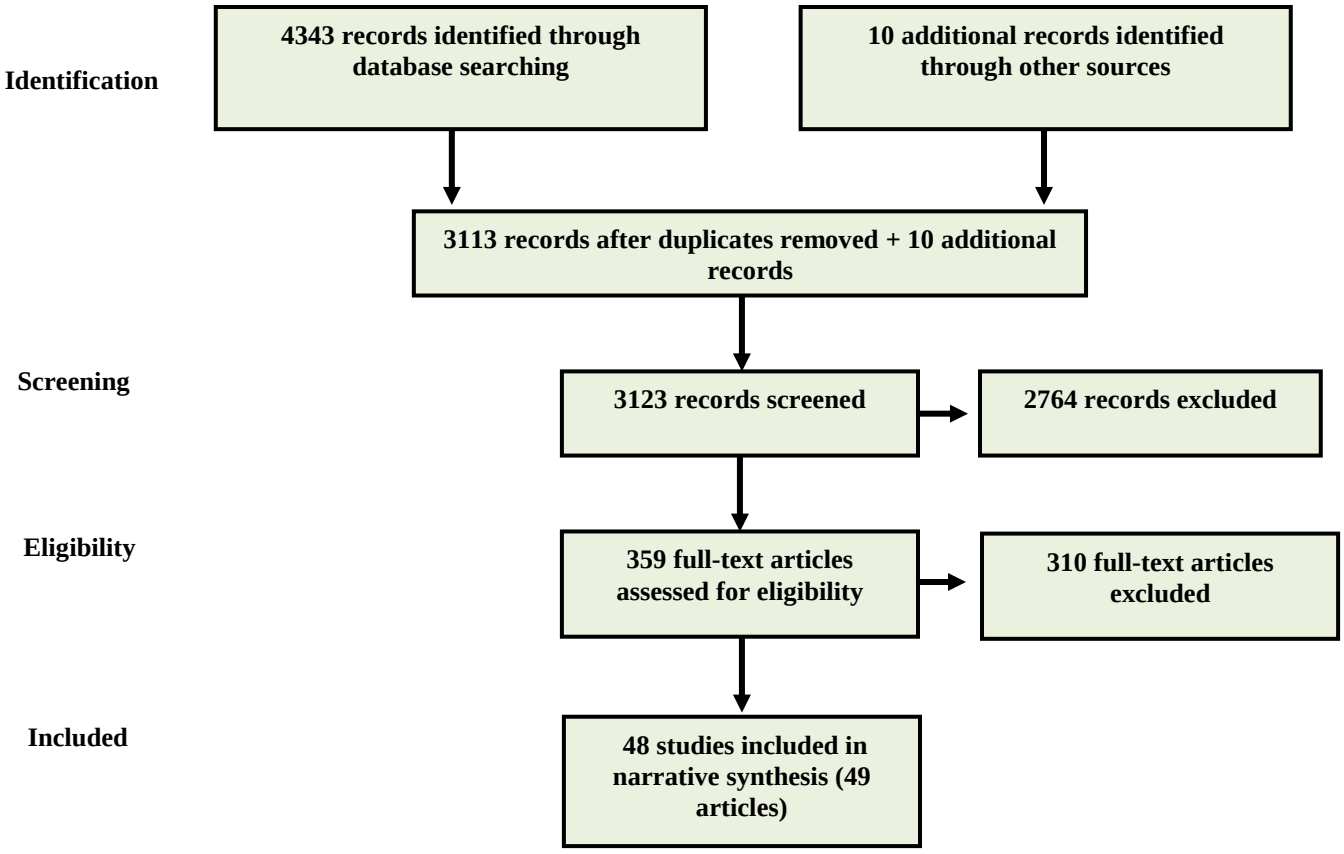
In October 2011, the electronic searches were run following rigorous development and optimisation of the complex search strategy (which included indexing and text terms, truncation and Boolean operators). Magnesium specific search strategies are detailed in **Appendix A: Magnesium.**

The methods for article screening, full text assessment, data extraction and validity assessment were as described in the Materials and Methods section above. Specific to magnesium, data not relevant to doses below the UL set at 250 mg/day were excluded (except when investigating balance and bioavailability factors).

Results

A total of 4343 records were identified through database searching, duplicate references (1230) were removed, resulting in 3113 references to screen. A further ten articles were identified by checking reference lists of pertinent reviews, included studies and current reports on DRVs. In total, 3123 articles were screened on the basis of title and abstract, resulting in 359 articles retrieved for full text assessment, and the final inclusion of 48 studies (49 articles) for this review which satisfied all inclusion criteria. These results are summarised in the PRISMA flow chart (**Figure 1**; Moher *et al*, 2009):

Figure 1. PRISMA flow chart



Of the 49 articles included, two publications reported on the same study group (Hadjistavri *et* al, 2010; Hatzistavri *et* al, 2009). Therefore, a total of 48 studies were included and were classified as: 1 SR (that included 27 studies); 1 meta-analysis (that included 27 balance studies); 5 depletion; 13 balance; 2 balance/ isotope; 5 isotope; 7 RCTs; 1 CT; and 13 cross-sectional studies. The studies included have been grouped and summarised under the following headings: cardiovascular risk factors (8); osteoporosis risk factors (2); magnesium metabolism (25); and concentration of magnesium in breast milk (13). The majority of studies were conducted in adult females (28) and mixed adult population groups (15), with only two studies conducted in children and two studies including only adult male participants. The systematic review included both children and adults. The included studies are summarised by primary endpoint, study type and population in **Table 1**. The study methodology, results and quality are described in detail in the sections below and summarised by endpoint in **Tables 2-5**.

Table 1. Summary of studies included [(a)]

Endpoint	Study type	Population
Cardiovascular risk factors (8)	Depletion (2) RCT (5) CT (1)	Adult, mixed (6) Adult, female (2)
Osteoporosis risk factors (2)	Depletion (1) RCT (1)	Adult, mixed (1) Adult, female (1)
Metabolism (25)	SR (1) Meta-analysis (1) Depletion (2) Balance (13) Balance/ isotope (2) Isotope (5) RCT (1)	Any (1) Adult, mixed (8) Adult, male (2) Adult, female (12) Children, mixed (1) Children, female (1)
Breast milk concentration (13)	Cross-sectional (13)	Adult, female (13)

(a): Number of studies included for each endpoint, study type and population is given in brackets

Validity

Validity was assessed for each study: 2 were assessed as being at low risk of bias, 4 at moderate risk of bias, and 42 at high risk of bias. The one systematic review included (Witowski *et al*, 2011) was assessed as being at high risk of bias. The study methodology claims to be based on the standard methodology developed for the EURopean micronutrient RECommendations Aligned (EURRECA) Network of Excellence (Hooper *et al*, 2009) and on the basis of this would be classified as of a higher quality, however the methodology does, in some instances, appear to deviate from this, for example, by using single data extraction during parts of the review process instead of duplicated data extraction. For depletion/ balance/ isotope studies, lack of information on the accuracy of the dose provided, or failure

to consider complete collection of excreta were the principal reasons for classification of high risk of bias. Controlled trials were considered as being at high risk of bias if they were not randomised correctly or did not adequately report the method of randomisation. Cross-sectional breast milk studies that did not sufficiently report magnesium dietary intake data were considered as being at high risk of bias.

CARDIOVASCULAR RISK FACTORS (TABLE 2)

Eight studies reported on the effect of magnesium on changes to blood pressure, heart rhythm, cholesterol, blood lipids, insulin resistance/ glucose metabolism, or other cardiovascular risk factors. Additionally Doyle *et al* (1999; **Table 3**) and five metabolic studies (**Table 4**) included some data on cardiovascular risk factors (Hunt *et al*, 1997; Lukaski and Nielsen, 2002; Nielsen and Milne, 2003; Nielsen, 2004b; Nielsen *et al*, 2007b). All studies were assessed as being at high risk of bias, other than Nielsen *et al* (2007b) which was assessed as being at moderate risk of bias. Studies tended to report on more than one risk factor.

Blood pressure

Eight studies (Borrello *et al*, 1996; Doyle *et al*, 1999; Ferrara *et al*, 1992; Hadjistavri *et al*, 2010; Hunt *et al*, 1997; Nielsen *et al*, 2007b; Rylander and Arnaud, 2004; Sacks *et al*, 1998) included some information on blood pressure, with five studies finding no significant changes to blood pressure, when supplementing subjects with up to 336 mg magnesium/day in comparison to a basal diet. Two studies (Borrello *et al*, 1996; Hadjistavri *et al*, 2010 [**Table 2**]) did find reductions in blood pressure in subjects supplemented with ~52-241 mg magnesium/day for 12 weeks, although Borrello only found a reduction in systolic blood pressure and not in diastolic or ambulatory blood pressure. Hunt *et al* (1997;**Table 4**) fed subjects a diet containing 109 mg magnesium/day with or without a 200 mg magnesium supplement. No significant differences in blood pressure between the basal and supplemented diets were reported. Blood pressure was 114/69 and 113/69 mm Hg for basal and supplemented diets respectively. Hadjistavri *et al* (2010) and Nielsen *et al* (2007b) were the only studies reporting blood pressure variables which did not use a randomised; or Latin-square design.

Heart rhythm

Four studies included information on changes to heart rhythm/ heart beat rates (**Table 2:** Klevay and Milne, 2002; **Table 4:** Hunt *et al*, 1997; Lukaski and Nielsen, 2002; Nielsen *et al*, 2007b). Three studies found evidence for a disruption in cardiac rhythm when magnesium intake was relatively low (~130 mg/day), but Hunt reported no interruption in normal sinus rhythm in subjects.

Cholesterol and blood lipids

Five studies (**Table 2:** Ferrara *et al*, 1992; Hadjistavri *et al*, 2010; **Table 4:** Nielsen and Milne, 2003; Nielsen, 2004b; Nielsen *et al*, 2007b) reported whether there were any changes in cholesterol or blood lipids. Hadjistavri found a reduction in total cholesterol, LDL cholesterol and triglycerides in subjects given dietary advice and supplemented with ~52 mg magnesium/day compared to a control group given only dietary advice. Nielsen (2003; 2004b; 2007b) found reduced total cholesterol/ LDL cholesterol when subjects were fed ~115 mg magnesium/day compared to subjects supplemented at higher levels (~320-410 mg total magnesium/day). Ferrara found no changes to blood lipids.

Insulin resistance/ glucose metabolism

Three studies reported on changes to glucose metabolism. Hadjistavri *et al* (2010) found a reduction in homeostasis model assessment-estimated insulin resistance (HOMA-IR), area under the curve for glucose and insulin, and fasting insulin levels, when subjects were supplemented with ~52 mg magnesium/day. Nadler *et al* (1993) found a reduction in insulin sensitivity when subjects were depleted with a liquid low magnesium diet (12 mg magnesium/day), but found no change in fasting serum glucose or insulin concentration. Nielsen *et al* (2007b) found the area under the curve for serum glucose was greater for subjects fed a basal diet of 101 mg magnesium/2000 kcal, compared to subjects fed the basal diet supplemented with 200 mg magnesium/day, but there was no change in the insulin response.

Platelet reactivity

Nadler *et al* (1992) fed subjects a liquid low magnesium diet (12 mg magnesium/day) for three weeks and found magnesium deficiency enhanced platelet aggregation and may therefore be a risk factor in vascular disease.

Quality of life

Borrello *et al* (1996) was the only study to include information on quality of life and found significant improvements to both health (e.g. reduced frequency of chest pain and improved respiratory function) and psychosocial activities (e.g. job and hobby satisfaction) in subjects supplemented with ~241 mg magnesium/day for 12 weeks compared to a placebo supplemented control group.

OSTEOPORSIS RISK FACTORS (TABLE 3)

Two studies were included which reported on the influence of magnesium on biomarkers of bone formation and bone resorption. The studies had different designs and found conflicting results. Doyle *et al* (1999) was a randomised controlled trial, conducted in adult females, which compared usual dietary intake (~275 mg magnesium/day) with usual dietary intake

supplemented with ~250 mg magnesium. Doyle reported no significant differences in bone biomarkers (serum calcium, osteocalcin, alkaline phosphatase, parathyroid hormone and urinary pyridinium). Fatemi *et al* (1991) depleted adults by feeding a liquid low magnesium diet (12 mg magnesium/day) for three weeks. Magnesium deficiency resulted in a significant fall in serum calcium and 1,25-dihydroxyvitamin D, and impaired secretion of parathyroid hormone. The authors concluded that magnesium deficiency may be a risk factor for osteoporosis. Both studies were assessed as being at high risk of bias. Four additional studies (**Tables 2 and 4**) included some data on magnesium losses and changes to mineral metabolism and bone biomarkers. Two studies found a significantly higher calcium balance under conditions of negative magnesium balance (Nielsen 2004b; Nielsen *et al*, 2007a). Two studies found no significant changes to ionised calcium, serum calcium or calcium balance (Klevay and Milne, 2002; Milne and Nielsen, 2000). There was little evidence for changes to other bone biomarkers.

METABOLISM (TABLE 4)

There were 25 studies included primarily concerned with magnesium status markers, balance and dietary influences on metabolism, with individual studies tending to report on more than one aspect of metabolism. Studies were assessed as being at high risk of bias (19), moderate risk of bias (4) and low risk of bias (2).

Status markers

Magnesium status was largely determined from urinary, faecal, serum and erythrocyte concentrations. Overall, data from all of the included studies in this review showed these markers to be responsive to dietary magnesium intake.

Witowski *et al* (2011) systematically assessed methods for measuring magnesium status in humans and undertook meta-analysis, thereby providing comparable data on status marker changes. This systematic review included a total of 27 studies (RCTs, CTs, before-after studies). Any individual studies in this systematic review which met our inclusion criteria have been data extracted separately (7 studies as cross-referenced in **Tables 2-4**: Fatemi *et al*, 1991; Lukaski and Nielsen, 2002; Nielsen and Milne, 2003; Nielsen 2004b; Nielsen *et al*, 2007a; 2007b; Sacks *et al*, 1998). Other trials did not meet our inclusion criteria (over the UL; non-EU approved forms; conducted in athletes).

Witowski found significant responses to magnesium intake for: serum/ plasma concentration (weighted mean difference [WMD]: 0.03 mmol/l, 95% CI 0.01, 0.06, $p<0.02$); erythrocyte magnesium concentration (WMD: 0.16 mmol/l, 95% CI 0.09, 0.22, $p<0.0001$); and urinary magnesium (WMD: 1.82 mmol/24 h, 95% CI 1.29, 2.36, $p<0.00001$). The heterogeneity in these results was relatively high: I^2 = 96%, 85% and 93% for serum/ plasma, erythrocyte and urinary magnesium respectively. The authors emphasise a paucity of data in this area and conclude that more high quality studies are required to determine biomarker responsiveness to

type, length and dose of supplementation for different population groups, particularly with regard to whole diets.

Magnesium balance

Hunt and Johnson (2006) pooled data from 27 balance studies conducted at the US Department of Agriculture, Agricultural Research, Grand Forks Human Nutrition Research Center, Grand Forks, ND. This study was assessed as being at moderate risk of bias. Any individual studies in this meta-analysis which met our inclusion criteria have been data extracted separately (6 studies as cross-referenced in **Table 4**: Hunt *et al*, 1995; 1997; Lukaski and Nielsen, 2002; Milne and Nielsen, 2000; Nielsen and Milne, 2003; Nielsen 2004b [three of these studies were also included in Witowski *et al*, 2011]). Other trials did not meet our inclusion criteria (conducted before January 1990; abstract only; insufficient information on magnesium intake and status; above the UL). Whilst only six individual studies met our study inclusion criteria, as a comprehensive meta-analysis, all data from Hunt and Johnson have been summarised here. Subjects were fed controlled Western diets (sometimes supplemented with test foods such as fructose, egg white) at magnesium intakes of between 84 and 598 mg/day, and the majority were not supplemented with magnesium. In the remaining studies included in the meta-analysis, the basal diet was supplemented with magnesium gluconate (except for one using magnesium citrate dibasic) in the range of 57-284 mg magnesium/day (with only one study supplementing at levels above the UL). Typically dietary treatments lasted six months. Magnesium output increased linearly with intake ($p=0.0001$), with neutral balance predicted at: 165 mg/day; or 2.36 mg/kg body weight/day, or 0.075 mg/kcal/day. Balance data were based on faecal and urinary excretion. Other losses were considered negligible (whole body surface 4.1 mg/day; phlebotomy 0.019 mg Mg/ml serum; and menstrual blood loss 2.3 mg/day). A strong homeostatic control of magnesium was evident, especially below zero balance. Age and gender were not found to significantly affect the predictions.

Other balance studies not included in the Hunt and Johnson meta-analysis, which reported on magnesium balance data in adults (n=10 in **Table 4**), found negative magnesium balance at intake levels of ~107-367 mg/day, and positive magnesium balance at levels of between ~176-520 mg magnesium/day.

Hunt *et al*, 1997 (included in the Hunt and Johnson meta-analysis) reported that an intake of 109 mg magnesium/day resulted in essentially zero magnesium balance in postmenopausal women (sweat loss of $\leq$15 mg/day was assumed). Griffin *et al*, 2008 (not included in the meta-analysis) predicted an intake of 52-78 mg magnesium/day would be needed to achieve a retention of 8-10 mg/day (estimated as needed for growth) in children aged 1-4 years. Andon *et al*, 1996 (not included in the meta-analysis) was the only other study in children (adolescent girls aged 11 years). This study reported that an intake of 6 mg/kg body weight/day (US RDA in this age group in 1996) would result in a magnesium balance $\geq$8.5 mg/day in 95% of the girls studied. Hunt *et al* (1997), Griffin *et al* (2008) and Andon *et al* (1996) were all assessed as being at high risk of bias.

Dietary influences on magnesium metabolism

Data regarding dietary influences on magnesium absorption or balance were difficult to interpret due to the wide variety of nutrients studied: vitamin B6 (1); boron (1); calcium (1); copper (3); fructose (2); meat (2); oxalate (1); phytic acid (2); and zinc (1). There were a total of 13 different studies altogether since one study (Nielsen and Milne, 2004a) included both zinc and copper information. The majority of studies (n=11) were assessed as being at high risk of bias; with no studies at low risk of bias and just two studies at moderate risk of bias (Hunt *et al*, 1995; Milne and Nielsen, 2000). Calcium had no significant effect on magnesium absorption or excretion (Andon *et al*, 1996). Changes in dietary boron had no significant effect on magnesium balance or the metabolic responses to dietary magnesium deprivation (Nielsen, 2004b). For copper, Klevay and Milne (2002) (**Table 2**) and Nielsen and Milne (2004a) reported no significant changes to magnesium metabolism. Nielsen and Milne (2003) found that copper had no significant effect on magnesium balance, although did have a limited effect on variables responding to magnesium deprivation, of note, erythrocyte magnesium was lower when dietary copper was lower. Significant reductions in magnesium absorption or balance were reported when magnesium was fed in conjunction with oxalate (Bohn *et al*, 2004a), phytic acid (Bohn *et al*, 2004b; Knudsen *et al*, 1996), or zinc (Nielsen and Milne 2004a). When dietary vitamin B6 was low, magnesium balance was reduced (Turnlund *et al*, 1992). Two studies reported on the effect of fructose. Ivaturi and Kies (1992) fed subjects a diet containing 154 mg magnesium/day (study A) or 341 mg magnesium/day (study B), in conjunction with either 60 g of sucrose or 60 g fructose. In study A, magnesium balance was negative for both treatments and was more negative when subjects were fed a fructose diet (-86.5) in comparison to a sucrose diet (-56.2). In study B, magnesium balance was positive, and there was no significant difference in magnesium balance between fructose (+64.8) or sucrose (+58.0) treatments. Milne and Nielsen (2000) found that higher dietary fructose resulted in a more positive magnesium balance when compared to a high starch diet. Hunt *et* al, 1995 and 1998 reported on the influence of meat on magnesium metabolism. Hunt *et al* (1995) found no significant difference in magnesium retention when comparing high and low meat diets. Hunt *et al* (1998) found apparent magnesium absorption was significantly lower from a lactoovovegetarian diet in comparison to a non vegetarian diet, although magnesium balance was unaffected.

Two additional studies, which were assessed as being at high risk of bias, measured magnesium absorption from mineral water. Sabatier *et al* (2002) found that when mineral water was fed in conjunction with a meal, magnesium absorption was significantly higher. Sabatier *et al* (2011) studied the effect of a bolus magnesium dose (2 x 750 ml mineral water) versus more frequent mineral water consumption during the day (7 x 212 ml), and found absorption of magnesium was higher when water was consumed more frequently.

BREAST MILK CONCENTRATION (TABLE 5)

All 13 breast milk studies included were assessed as being at high risk of bias. The studies report cross-sectional sample data from 1-380 days of lactation. Dengel *et al* (1994; **Table 4**),

a balance study assessed as being at high risk of bias, also reported breast milk data. The mean concentration of magnesium from all breast milk studies ranged from 23-47 mg/l. Variations are likely due to different analytical techniques employed within studies and due to differences in dietary patterns between countries (Parr *et al*, 1991).

Maternal magnesium intake was only reported in two studies, both using 3-day dietary records (**Table 5:** Rakicioglu *et al*, 2006; Tanzer and Sunel, 1991). Mean magnesium intake varied from 219-405 mg/day (under conditions of usual dietary intake). There were too few data to establish a correlation between magnesium intake and breast milk magnesium concentration. Similarly, there was no clear correlation between stage of lactation and breast milk magnesium concentration. Hunt *et al* (2005; **Table 5**) found there was a relatively wide variation between subjects at a given stage of lactation. Dengel *et al* (1994) provided a controlled diet to lactating and non-lactating women and found that lactating women excreted less urinary magnesium than never-pregnant women ($p<0.05$), which might be a compensatory mechanism for magnesium losses in breast milk.

Conclusions

Articles from Janaury 1990 to October 2011 have been systematically searched and reviewed using a standard protocol, tailored for the specific issues relevant to magnesium, with the aim of collating and assessing the body of evidence for magnesium relevant to setting DRVs.

A total of 48 studies met the inclusion criteria. The majority of studies included reported on magnesium metabolism and magnesium concentration within breast milk. Health endpoints included focused on cardiovascular and osteoporosis risk factors, however the data were fragmented and inconsistent. With regard to status markers, those that responded well to magnesium intake included serum/ plasma, erythrocyte and urinary magnesium concentrations. A meta-analysis conducted in adults predicted that an intake of 165 mg magnesium/day is required to maintain zero magnesium balance. Regarding data of relevance to DRV setting for infants, mean magnesium concentration in breast milk was found to vary from 23-47 mg/l.

The majority of studies included were assessed as being at high risk of bias (42), with the remainder of studies at moderate (4) and low risk of bias (2). Study data were largely restricted to adults and females in particular. Overall, data to potentially use for the setting of DRVs for magnesium were limited.

Table 2. Cardiovascular risk factors

Reference	Study type	n	Age	Sex	Intervention and control or exposure	Dietary intake estimation	Follow up time	Results: effect, mean, SD, RR/OR/HR confidence interval, etc	Overall results	Risk of bias
Borrello G *et al*, 1996	RCT	83	Placebo group: 49 ± 5years Mg group: 51 ± 7 years	Placebo group: 14M & 27F Mg group: 16M & 26F	i) Placebo group (n=41): placebo tablet/capsule (not defined) daily for 12 weeks ii) Mg group (n=42): 200 mg Mg oxide (19.86 mEq elemental Mg) daily for 12 weeks	Not reported	12 weeks	Systolic BP (mean ± SD) significantly decreased at 12 weeks compared to baseline, and compared to placebo: 148.5 ± 7.1 at 12 weeks, compared to 155 ± 13 at baseline p<0.01 148.5 ± 7.1 at 12 weeks, compared to 155.2 ± 8.2 (placebo group at 12 weeks) p<0.01 Serum Mg significantly increased at 12 weeks, compared to baseline: 1.0 ± 0.2 at 12 weeks, compared to 0.9 ± 0.2 at baseline p<0.001 1.0 ± 0.2 at 12 weeks, compared to 1.0 ± 0.4 (placebo group at 12 weeks) Urinary Mg significantly increased at 12 weeks, compared to placebo: 5.8 ± 1.2 at 12 weeks, compared to 4.3 ± 1.0 at baseline	Mg oxide significantly reduced systolic blood pressure (p<0.01) and positively influenced quality of life (including a reduction in frequency of chest pain; improvements in respiratory function; increase in physical activity). No reduction in diastolic blood pressure or 24 h-ambulatory blood pressure were observed	High

Reference	Study type	n	Age	Sex	Intervention and control or exposure	Dietary intake estimation	Follow up time	Results: effect, mean, SD, RR/OR/HR confidence interval, etc	Overall results	Risk of bias
								5.8 ± 1.2 at 12 weeks, compared to 4.2 ± 2.6 (placebo group at 12 weeks) p<0.05 <u>Quality of life scores</u> significantly lowered (indicating an improvement) at 12 weeks compared to baseline, and compared to placebo: 67.58 ± 5 points at 12 weeks, compared to 73.58 ± 6 at baseline p<0.05 67.58 ± 5 points at 12 weeks, compared to 73.23 ± 8 (placebo group at 12 weeks) p<0.05		
Ferrara L *et al*, 1992	RCT	14	40-60 years	8M & 6F	i) Magnesium pidolate (15 mmol/d) (n=7) ii) Placebo (n=7)	Not reported	6 months	Supine and upright BP changes: Mg group: -4.5% (supine); -7.7% (upright) placebo group: -10.7% (supine); -4.3% (upright) Peripheral resistances were 14.7 ± 4 and 9.8 ± 2 PRU	Mg treatment did not significantly affect blood pressure Changes to total cholesterol, HDL cholesterol and triglycerides were not significant	High

Reference	Study type	n	Age	Sex	Intervention and control or exposure	Dietary intake estimation	Follow up time	Results: effect, mean, SD, RR/OR/HR confidence interval, etc	Overall results	Risk of bias
								before and after Mg supplementation respectively. The trend for a reduction in forearm peripheral resistance was not significant Urinary changes were significant ($p<0.01$) Mg group: increase from 5.3 ± 2 to 7.7 ± 2 mmol/24 h Placebo group: there was no difference (5.1 ±1 to 5.2 ±2). There were no significant changes in serum Mg		
Hadjistavri L *et al*, 2010; Hatzistavri L *et al*, 2009	CT	48	Control group: 46.9 ± 8.7 years Mg group: 45.3 ± 10.1 years	Control group: 15M, 9F Mg group: 15M & 9F	i) Dietary advice (n=24) ii) Dietary advice + 600 mg Mg pidolate (n=24)	Not reported	12 weeks	<u>At study end (mean ± SD):</u> <u>Fasting insulin (pmol/l):</u> Mg: 10.11 ± 3.2 Control: 9.93 ± 3.4 ($p<0.05$) <u>HOMA-IR index (insulin resistance):</u> Mg: 2.31 ± 0.7 Control: 2.24 ± 0.8 ($p<0.05$) <u>Total cholesterol (mg/100ml):</u> Mg: 200.9 ± 34.1 Control:	HOMA-IR (and AUC for glucose, AUC for insulin and fasting insulin levels), triglycerides, total cholesterol and LDL cholesterol were significantly reduced in the Mg group by 12 weeks follow-up compared to the control	High

Reference	Study type	n	Age	Sex	Intervention and control or exposure	Dietary intake estimation	Follow up time	Results: effect, mean, SD, RR/OR/HR confidence interval, etc	Overall results	Risk of bias
								229.1 ± 29.6 (p<0.05) <u>LDL cholesterol (mg/100ml):</u> Mg: 124.5 ± 32.1 Control: 160.4 ± 29.4 (p<0.001) <u>HDL cholesterol (mg/100ml):</u> Mg: 56.4 ± 12.4 Control: 42.0 ± 6.2 (p<0.001) <u>Triglycerides (mg/100ml):</u> Mg: 99.8 ± 42.3 Control: 133.3 ± 28.6 (p<0.001) <u>24 h ambulatory BP:</u> SBP (mm Hg): Mg: 141.1 ± 4.1 Control: 143.4 ± 5.4 (p<0.001) DBP (mm Hg): Mg: 88.7 ± 2.9 Control: 89.5 ± 3.8 (p<0.001) <u>Serum Mg (mg/100ml):</u> Mg: 2.44 ± 0.2 Control: 2.1 ± 0.2 (p<0.01) <u>24 h urinary Mg (mg/l):</u> Mg: 178.4 ± 89.8 Control: 62.1 ± 34.9 (p<0.001)	group (along with a parallel increase in HDL cholesterol) p<0.05 Overall 24 h changes in SBP between baseline and 12 weeks in the Mg and control groups were: -5.6 ± 2.7 mm Hg versus -1.3 ± 2.4 mm Hg p<0.001 Overall 24 h changes in DBP between baseline and 12 weeks in the Mg and control groups were: -2.8 ± 1.8 mm Hg versus -1.0 ± 1.2 mm Hg p=0.002 Serum and urinary Mg levels were significantly increased in the intervention group but not in the control group	

Reference	Study type	n	Age	Sex	Intervention and control or exposure	Dietary intake estimation	Follow up time	Results: effect, mean, SD, RR/OR/HR confidence interval, etc	Overall results	Risk of bias
Klevay L and Milne D, 2002	RCT	22	47-78 years	F	Rotating menu: i) Low Mg diet provided 100 mg/d Mg (supplemented with placebo capsules). Mean Mg intake: 130 mg ii) High Mg diet was basal diet supplemented with 200 mg/d Mg as Mg gluconate capsules. Mean Mg intake: 411 mg (Additionally one-half of women were supplemented with 1 mg/d copper and the rest with 3 mg copper)	Dietary analysis	81 day test periods	<u>At study end (means ± SEM):Serum Mg, mmol/l:</u> Low Mg diet: 0.83 ± 0.007; High Mg diet: 0.86 ± 0.007 ($p<0.005$) <u>Erythrocyte Mg, µmol/g Hb:</u> Low Mg diet: 6.01 ± 0.058; High Mg diet: 6.21 ± 0.058 ($p<0.025$) <u>Ultrafilterable Mg, mmol/l:</u> Low Mg diet: 0.57 ± 0.003; High Mg diet: 0.59 ± 0.003 ($p<0.03$) <u>Urine Mg, mmol/d:</u> Low Mg diet: 2.88 ± 0.12; High Mg diet: 5.68 ± 0.12 ($p<0.0001$) <u>Supraventricular beats (% of beats):</u> Low Mg diet: 0.074 (0.034-0.128); High Mg diet: 0.042 (0.014-0.085) $p<0.02$ <u>Ventricular beats (% of beats)</u> Low Mg diet: 0.125 (0.038-0.262); High Mg diet: 0.073 (0.012-0.184)	Authors only present results for magnesium as copper did not change magnesium metabolism or other variables (Cu was administered as a diet low in Mg will also be low in Cu) Serum (3.5%), erythrocyte (3.3%), and ultrafilterable Mg (4.2%) concentrations were significantly lower when the amount of dietary Mg was lower. Urinary Mg excretions were 49% lower Cardiac rhythm was disrupted when Mg intake was lower. Supraventricular beats were more frequent ($p<0.020$) and the sum of ventricular and	High

Reference	Study type	n	Age	Sex	Intervention and control or exposure	Dietary intake estimation	Follow up time	Results: effect, mean, SD, RR/OR/HR confidence interval, etc	Overall results	Risk of bias
								Sum of above: Low Mg diet: 0.274 (0.139-0.453); High Mg diet: 0.160 (0.063-0.303) p<0.015	supraventricular beats was significantly higher (p<0.015) Changes in K, ionized calcium, serum calcium and parathyroid hormone concentrations were not significant	
Nadler J *et al*, 1992	Depletion	16	N/R	11M & 5F	Constant liquid low-Mg diet (12 mg Mg/d) for 3 weeks	Fixed diet and dietary analysis	3 weeks	Low Mg diet compared to baseline: Significantly less U46619 and ADP (aggregating agents) were needed to induce maximal platelet aggregation in Mg-deficient subjects (p<0.02) Reduced serum Mg (1.9 ± 0.1 to 1.5 ± 0.07 mEq/l) p<0.01 Reduced intracellular free RBC Mg (194 ± 10 to 127 ± 9 µM) p<0.01	Mg deficiency may increase platelet reactivity and therefore be a risk factor in vascular disease (particularly within diabetic subjects)	High

Reference	Study type	n	Age	Sex	Intervention and control or exposure	Dietary intake estimation	Follow up time	Results: effect, mean, SD, RR/OR/HR confidence interval, etc	Overall results	Risk of bias
Nadler J *et al*, 1993	Depletion	12	N/R	M & F	i) Liquid diet supplemented with 400 mg $MgCl_2$/d for 1 week ii) then constant liquid low-Mg diet (12 mg Mg/d) for 3 weeks	Fixed diet and dietary analysis	4 weeks	<u>Low Mg diet compared to week 1:</u> Increased urinary thromboxane (272 ± 30 to 376 ± 90 ng/g creatinine) p<0.02 Increased plasma aldosterone (18 ± 2 to 37 ± 5 ng/dL) p<0.02 Reduced insulin sensitivity (3.69 ± 0.6 to 2.75 ± 0.5/min/microunit/ml x 10-4) p<0.03 Reduced serum Mg (0.78 ± 0.08 to 0.53 ± 0.08 mmol/l) p<0.01 Reduced intracellular free RBC Mg (186 ± 10 to 127 ± 9 mM) p<0.01	Changes in thromboxane and aldosterone levels, and a reduction in insulin sensitivity suggest Mg deficiency may be a risk factor for insulin resistance and vascular disease Fasting serum glucose and insulin concentration were not affected	High
Rylander R and Arnaud M, 2004	RCT	55	45-64 years	M & F	Random allocation to 3 waters, labelled by letter: A) Valvert® water (low in minerals: 2 mg Mg/l) B) Distilled water +	Not reported	4 weeks	<u>Systolic BP</u> Water A: Before: 151.9 ± 9.8; After: 148.3 ± 12.4 Water B: Before: 148.3 ± 10.5; After: 147.9 ± 11.5 Water C: Before: 156.8 ± 15.9; After: 150.4 ± 15.5	Significant reductions in systolic and diastolic BP were seen after consumption of water C only. There was no significant effect on BP after consumption of	High

Reference	Study type	n	Age	Sex	Intervention and control or exposure	Dietary intake estimation	Follow up time	Results: effect, mean, SD, RR/OR/HR confidence interval, etc	Overall results	Risk of bias
					$MgSO_4$ (82.3 mg Mg/l) C) Contrex® water (natural mineral water: 84 mg Mg/l)			(p=0.017) <u>Diastolic BP</u> Water A: Before: 90.1 ± 4.4; After: 89.8 ± 5.0 Water B: Before: 90.4 ± 4.2; After: 90.9 ± 6.6 Water C: Before: 91.7 ± 6.3; After: 89.1 ± 8.0 (p=0.02) <u>Urinary Mg (mmol/l)</u> Water A (n=18): Before: 0.25 ± 0.08; After: 0.26 ± 0.07 Water B (n=18): Before: 0.28 ± 0.06; After: 0.34 ± 0.09 (p=0.009) Water C (n=19): Before: 0.30 ± 0.07; After: 0.35 ± 0.09 (p=0.019)	the water which was high in Mg only (water B) Subjects consuming waters B and C had significantly higher amounts of Mg in urine post-intervention. There was no difference in amounts of Mg in urine post-intervention in the group consuming water A There were no significant effects of the waters on serum levels of Mg for any of the groups	
Sacks F *et al*, 1998 (Included within Witowski *et al*,	RCT	300	39 ± 5 years	F	Groups of relevance to review: 1) Magnesium lactate 336 mg/d (n=50)	FFQ	16 weeks	<u>Dietary intake (mg/d)</u> Mg group at study end: 582 ± 87 Placebo group at study end: 229 ± 76	<u>Average change in 24 h ambulatory BP versus placebo</u>: Systolic: -0.9 (-2.6, 0.8), p=0.29 (NS) Diastolic: -0.7 (-2.2,	High

Reference	Study type	n	Age	Sex	Intervention and control or exposure	Dietary intake estimation	Follow up time	Results: effect, mean, SD, RR/OR/HR confidence interval, etc	Overall results	Risk of bias
2011)					2) Placebo (n=103)			<u>Change in 24 h ambulatory BP from baseline (mm Hg)</u> Mg group at midpoint: systolic -0.7 ± 5.8, diastolic -0.5 ± 4.5 Placebo group at midpoint: systolic 0.3 ± 5.3, diastolic 0.2 ± 4.0 Mg group at endpoint: systolic -0.5 ± 4.8, diastolic -0.5 ± 4.4 Placebo group at endpoint: systolic 0.4 ± 5.6, diastolic 0.3 ± 4.8 <u>Urinary excretion (mmol/24 h)</u> Mg group after treatment: 6.2 ± 2.0 Placebo group after treatment: 3.8 ± 1.4	0.8), p=0.32 (NS) <u>Urinary Mg excretion change from baseline to end (mmol/24 h)</u>: Mg group: 2.1 ± 1.7 (p<0.01 within group; p<0.01 vs placebo) Placebo group: -0.1 ± 1.6 (NS)	

(a): Cardiovascular data from Doyle *et al*, 1999 were included in **Table 3**; Hunt *et al*, 1997; Lukaski and Nielsen, 2002; Nielsen and Milne, 2003; Nielsen 2004b; Nielsen *et al*, 2007b were included **in Table 4**

Table 3. Osteoporosis risk factors

Reference	Study type	n	Age	Sex	Intervention and control or exposure	Dietary intake estimation	Follow up time	Results: effect, mean, SD, RR/OR/HR confidence interval, etc	Overall results	Risk of bias
Doyle L *et al*, 1999	RCT	26	23.0 ± 2.0 years (20-28)	F	i) Usual dietary intake + placebo: 11.3 ± 3.6 mmol (275 mg) Mg/d ii) Usual dietary intake + 10.3 mmol (250 mg) Mg as Mg hydroxide: 22.7 ± 4.6 mmol (525 mg) Mg/d	Food records	28 day test periods	There were no significant differences in serum Ca or biomarkers of bone formation (serum osteocalcin, bone-specific alkaline phosphatase, parathyroid hormone); or biomarkers of bone resorption (cross-links of collagen-urinary pyridinium) Biomarker changes post diet (means ± SEM): Erythrocyte Mg (mmol/l): Placebo: 2.31 ± 0.44 Mg group: 2.70 ± 0.39 Urine Mg (mmol/mmol creatinine): Placebo: 0.28 ± 0.09 Mg group: 0.38 ± 0.11 Serum Mg (mmol/l): Placebo: 0.76 ± 0.09 Mg group: 0.77 ± 0.11	Erythrocyte Mg content in the group receiving Mg supplements was significantly greater than the placebo ($p=0.02$) Urinary excretion of Mg/Cr increased significantly by ~36% ($p<0.001$). There were no significant differences in other biomarkers, BP or bone markers when subjects switched from the unsupplemented to Mg supplemented diet	High

Reference	Study type	n	Age	Sex	Intervention and control or exposure	Dietary intake estimation	Follow up time	Results: effect, mean, SD, RR/OR/HR confidence interval, etc	Overall results	Risk of bias
Fatemi S *et al*, 1991 (Included within Witowski *et al*, 2011)	Depletion	26	18-48 years	22M & 4F	Liquid Mg-low diet (12 mg Mg/d). The diet was replete in all other macro- and micronutrients (providing 36 Cal/kg, 0.8 g protein/kg body weight)	Fixed diet	21 days	Means ± SEM: Serum Ca (mmol/l) Pre-diet: 2.36 ± 0.02 Post-diet: 2.31 ± 0.03 ($p<0.05$) 1, 25 dihydroxyvitamin D (pmol/l) Pre-diet: 55 ± 3.6 Post diet: 43 ± 3.1 ($p<0.05$) Immunoreactive PTH (% change): -0.6 ± 9.5 (NS) Urinary Mg/ creatinine ratio Pre-diet: 0.053 ± 0.006 Post-diet: 0.016 ± 0.006 ($p<0.001$) Serum Mg (mmol/l) Pre-diet: 0.80 ± 0.01 Post-diet: 0.61 ± 0.02 ($p<0.001$) Erythrocyte Mg (µmol) Pre-diet: 205 ± 10 Post-diet: 162 ± 7 ($p<0.001$) Mg retention (%) Pre-diet: 11 ± 4 Post-diet: 62 ± 4 ($p<0.001$)	Mg deficiency can be induced within 3 weeks, shown by a significant fall in serum and erythrocyte Mg and an increase in Mg retention. Mg deficiency resulted in a fall in serum calcium and 1,25-dihydroxyvitamin D. The changes were dependent only on the fall in serum Mg. PTH secretion was impaired shown by a fall or no change in serum PTH despite a fall in the serum Mg and Ca. The fall in 1,25 dihydroxyvitamin D may be due to both the decrease in PTH secretion and a renal resistance to	High

Reference	Study type	n	Age	Sex	Intervention and control or exposure	Dietary intake estimation	Follow up time	Results: effect, mean, SD, RR/OR/HR confidence interval, etc	Overall results	Risk of bias
									PTH. Mild Mg depletion can impair mineral metabolism and may be a risk factor for osteoporosis	

(a): Bone metabolism data from Klevay and Milne, 2002 were included in **Table 2**; Milne and Nielsen, 2000; Nielsen 2004b; Nielsen *et al*, 2007a were included in **Table 4**

Table 4. Metabolism

Reference	Study type	n	Age	Sex	Intervention and control or exposure	Dietary intake estimation	Follow up time	Results: effect, mean, SD, RR/OR/HR confidence interval, etc	Overall results	Risk of bias
Andon M *et al*, 1996	Balance	26	11.3 years	F	1) Basal diet + placebo tablet (n=13). Dietary Mg 177 ± 18 mg/d 2) Basal diet + 1000 mg/d Ca (n=13). Dietary Mg 175 ± 16 mg/d	Duplicate diet (3 day dietary records at baseline)	14 days (7 day sample collection)	Mg Balance (mg/d ±SD): Basal diet: 19 ± 25 Basal + Ca: 22 ± 15 Net Mg absorption (mg/d ±SD): Basal diet: 89 ± 18 Basal + Ca: 96 ± 14 Mg absorption (% of intake): Basal diet: 50 ± 9 Basal + Ca: 55 ± 8	Ingestion of an additional 1000 mg/d Ca had no significant effect on net absorption of Mg or on faecal or urinary Mg excretion. Thus, Mg balance was not altered by the high- or low-calcium diet. Mg intake was significantly correlated with Mg absorption (r=0.723, p<0.001) Mg intake was significantly correlated with Mg balance (r=0.511, p=0.008) Mg intake of 6 mg/kg body weight/d would result in Mg balance	High

Reference	Study type	n	Age	Sex	Intervention and control or exposure	Dietary intake estimation	Follow up time	Results: effect, mean, SD, RR/OR/HR confidence interval, etc	Overall results	Risk of bias
									of ≥8.5 mg/d in 95% of the girls	
Bohn T *et al*, 2004a	Isotope	9	23 ± 1 years	M & F	Test meals: i) High Oxalate: pureed spinach (300 g; 1.12 ± 0.09 mmol/100g native Mg content; 6.6 mmol oxalate) mixed with stable isotope ^{25}Mg (0.66 mmol) provided with phytate-free white bread rolls (Total Mg content of test meal: 4.98 ± 0.01 mmol) ii) Low oxalate: pureed Kale (300 g; 0.89 ± 0.01 mmol/100g native Mg content; 0.1 mmol oxalate) mixed with stable isotope ^{26}Mg (1.19 mmol) provided with phytate-free white bread rolls (Total Mg content of test meal: 4.82 ± 0.05 mmol)	Not reported	Complete faecal collection	Mg absorption from spinach test meal (%): 26.7 ± 10.4 (significantly lower than absorption from kale test meal, p=0.01) Mg absorption from kale test meal (%): 36.5 ± 11.8 Absorption ratios (spinach test meal/kale test meal): 0.73 ± 0.19	Mean fractional apparent absorption of Mg from spinach (high oxalate) test meal was ~35% lower than from the kale (low oxalate) test meal	High
Bohn T *et al*, 2004b	Isotope	17	Study 1: 27 ± 12	M & F	Test meals consisting of 200g phytic acid-free white wheat bread (all test meals	Not reported	Complete faecal	Addition of 1.49 mmol phytic acid/200 g bread reduced fractional apparent absorption	Mean fractional apparent Mg absorption was ~60%	High

Reference	Study type	n	Age	Sex	Intervention and control or exposure	Dietary intake estimation	Follow up time	Results: effect, mean, SD, RR/OR/HR confidence interval, etc	Overall results	Risk of bias
			years Study 2: 24 ± 2 years		standardised to 3.6 mmol total Mg): <u>Study 1:</u> Test meal A: 1.5 ± 0.02 mmol phytic acid (similar to level in wholemeal bread) labelled with 0.65 mmol ^{25}Mg Test meal B: 0 mmol phytic acid labelled with 1.12 mmol ^{26}Mg <u>Study 2:</u> Test meal A: 0.75 ± 0.002 mmol phytic acid (similar to level in brown bread) labelled with 0.65 mmol ^{25}Mg Test meal B: 0 mmol phytic acid labelled with 1.12 mmol ^{26}Mg		collection	from 32.5 ± 6.9% to 13.0 ± 6.9 % ($p<0.0005$) Addition of 0.75 mmol phytic acid/200 g bread reduced fractional apparent absorption from 32.2 ± 12.0 % to 24.0 ± 12.9 % ($p<0.01$)	lower when phytic acid was at a level comparable to wholemeal bread (1.49 mmol/200 g), and ~25% lower when phytic acid was at a level comparable to brown bread (0.75 mmol/200 g). The inhibiting effect of phytic acid on fractional apparent Mg absorption was dose dependent ($p<0.005$)	
Dengel J *et al*, 1994	Balance	19	21-34 years	F	Rotating menu with controlled Mg content: 215 ± 8 mg/d, fed to: i) Lactating women (n=6) ii) Non-lactating women (n=6) iii) Never pregnant women	Duplicate diet (7 day dietary records at baseline)	20 days	<u>Urine Mg excretion (mmol/d, mean ± SEM):</u> L group (n=6): 2.10 ± 0.35 $p<0.05$ compared to NP group NL group (n=5): 2.66 ± 0.24 NP group (n=6): 3.45 ± 0.37	Lactating women excreted less urinary Mg than never-pregnant women $p<0.05$ For lactating women,	High

Reference	Study type	n	Age	Sex	Intervention and control or exposure	Dietary intake estimation	Follow up time	Results: effect, mean, SD, RR/OR/HR confidence interval, etc	Overall results	Risk of bias
					(n=7)			Faecal Mg excretion (mmol/d, mean ± SEM): L group (n=6): 4.90 ± 0.57 NL group (n=6): 3.68 ± 0.5 NP group (n=7): 4.47 ± 0.24 Apparent Mg absorption (%): L group (n=6): 46 ± 6 NL group (n=6): 59 ± 6 NP group (n=7): 50 ± 3 Mg balance (mmol/d): values for L group include breast milk losses; values for all groups do not include sweat or dermal Mg losses: L group (n=6): 0.84 ± 0.69 NL group (n=6): 1.87 ± 0.58 NP group (n=7): 1.61 ± 0.42	breast milk Mg concentration was 1.37 ± 0.01 mmol/l (33.3 ± 0.24 mg/l); infant milk intake was 761 ± 83.5 ml/d. This resulted in 1.04 ± 0.06 mmol Mg/d secreted into breast milk Significant negative correlation determined between pre-study Mg intake and Mg balance (r= -0.68, p<0.01) All subjects (except 1 lactating women) were in positive balance when consuming ~9 mmol Mg	
Desbiens N *et al*, 1992	RCT	48	22-76 years	21M & 27F	Of relevance to review: Control group (n=14): 40 mg pyridoxine (as pyridoxine hydrochloride)	Not reported	1 month-results compared	Serum Mg (mmol/l) ± SD: Baseline: placebo: 0.89 ± 0.06; group A: 0.84 ± 0.05 Post-supplementation: placebo:	There were no significant changes in serum Mg or intracellular Mg	High

Reference	Study type	n	Age	Sex	Intervention and control or exposure	Dietary intake estimation	Follow up time	Results: effect, mean, SD, RR/OR/HR confidence interval, etc	Overall results	Risk of bias
					Group A (n=18): 362 mg Mg as Mg oxide plus 40 mg pyridoxine		over a year	0.86 ± 0.07; group A: 0.81 ± 0.06 Intracellular mononuclear Mg (fmol/cell): Baseline: placebo: 2.87 ± 1.37; group A: 2.46 ± 0.68 Post-supplementation: placebo: 3.21 ± 0.99; group A: 2.93 ± 1.07 Higher in the months of Aug, Sept and Oct, than in the months of Feb, May, June and July (p=0.05 overall)	overall	
Griffin I *et al*, 2008	Balance/ isotope	30	29.6 ± 10.4 months (12-48)	14M & 16F	7 day dietary plan to reflect usual Mg intake. Subsequently: 5 mg ^{25}Mg infused intraveneously and 10 mg ^{26}Mg provided in 30 g apple juice (5 mg with a scheduled breakfast and 5 mg with lunch)	Dietary records pre-study, and mineral analysis during study	48-120 hours	Mg dietary intake (mg/d) ± SD: 106 ± 25 Urinary Mg (mg/d): 24 ± 14 Endogenous faecal Mg excretion (mg/d): 12 ± 5 Urinary and faecal Mg excretion were not significantly correlated to Mg intake Mg balance (mg/d): 19 ± 19 Mg absorption (%): 51.8 ± 8.3; (mg/d): 54 ± 13 Fractional Mg absorption was	Based on estimated Mg retention data, a retention of zero would be achieved at an intake of 45 mg/d, and retentions of 8-10 mg/d (estimated as needed for growth in 1-4 year olds), would require intakes of 71-78 mg/d. When data is limited only to	High

Reference	Study type	n	Age	Sex	Intervention and control or exposure	Dietary intake estimation	Follow up time	Results: effect, mean, SD, RR/OR/HR confidence interval, etc	Overall results	Risk of bias
								weakly but significantly negatively correlated with Mg intake (y=0.65-0.001x, p=0.0383; r^2=0.144) Absolute Mg absorption (product of fractional absorption and intake) was significantly positively correlated with Mg intake (y=12.71+0.389x; r^2=0.566, p<0.0001) Mg retention was negative in only 4 subjects (13%) and was significantly postively correlated to Mg intake (y= -13.58 + 0.302x, r^2 = 0.157, p=0.0304)	subjects for direct faecal measurements, zero retention would be achieved at ~30 mg/d and retention of 8-10 mg/d would occur at 52-57 mg/d	
Hunt C and Johnson L, 2006	Meta-analysis of balance studies	243 27 (a)	M: 28.1 ± 8.1 years F: 51.3 ± 17.4 years	93M & 150F	Rotating menu: (western diets, sometimes supplemented with test foods e.g. fructose, egg white drinks etc). Intakes ranged from 84-598 mg Mg/d. In most studies, the basal diet was not supplemented with Mg. In the remainder, the basal diet was supplemented with Mg gluconate (except for one	Duplicate diet	Typically 6 month test periods (18 days as a minimum)	Mg output increased linearly with intake (P=0.0001), with neutral balance predicted at: 165 mg/d (Y = 19.8 + 0.880 M, 95% PI: 113, 237 mg/d) 2.36 mg/kg body weight (Y = 0.306 + 0.870 M, 95% PI: 1.58, 3.38 mg/kg) 0.075 mg/Kcal/d (Y = 0.011 + 0.857 M, PI: 0.05, 0.11 mg/Kcal/d) Age and gender were not found	Strong homeostatic control of Mg, especially below neutral Mg balance (predicted at 165 mg/d)	Moderate

Reference	Study type	n	Age	Sex	Intervention and control or exposure	Dietary intake estimation	Follow up time	Results: effect, mean, SD, RR/OR/HR confidence interval, etc	Overall results	Risk of bias
					supplemented with Mg citrate dibasic) in the range of 57-284 mg Mg/d (with only 1 study over the UL)			to significantly affect the predictions Balance data were based on faecal and urinay output. Other losses were considered negligible (whole body surface 4.1 mg/d; phlebotomy 0.019 mg Mg/ml serum; and menstrual 2.3 mg/d)		
Hunt J *et al*, 1995 (Included within Hunt and Johnson, 2006)	Balance	14	62.9 ± 6.1 years (51-70)	F	Rotating menu: i) High meat diet with 289 g meat (268 ± 26 Mg mg/d) ii) Low meat diet with 38.5 g meat (214 ± 23 Mg mg/d) iii) Low meat diet with mineral supplements (257 ± 32 Mg mg/d). Mineral supplements were 748 mg K, 594 mg P, 3.3 mg Fe, 55 mg Mg, 5.5 mg Zn	Fixed diet and duplicate diet analysis	7 week test periods	<u>i) High Meat group</u> Urine: 89.9 mg/d (34 as % of diet) Faeces: 230 mg/d (86 as % of diet) Balance: -52 mg/d (-20 as % of diet) <u>ii) Low Meat group</u> Urine: 81.2 mg/d (38 as % of diet) Faeces: 174 mg/d (81 as % of diet) Balance: -40 mg/d (-19 as % of diet) <u>iii) Supplemented low meat</u> Urine: 80.8 mg/d (31 as % of	Mg retention was negative when Mg intakes were 210-270 mg/d. There was no difference in Mg retention between the three diets Urinary Mg excretion (mg/d) was significantly greater in the high-meat diet, when compared to the low-meat supplemented diet ($p<0.02$)	Moderate

Reference	Study type	n	Age	Sex	Intervention and control or exposure	Dietary intake estimation	Follow up time	Results: effect, mean, SD, RR/OR/HR confidence interval, etc	Overall results	Risk of bias
								diet) Faeces: 218 mg/d (85 as % of diet) Balance: -42 mg/d (-17 as % of diet)	Faecal Mg excretion (mg/d) was significantly greater in the high meat and low meat, supplemented diets, when compared to the low-meat diet (p=0.0001)	
Hunt C *et al*, 1997 (Included within Hunt and Johnson, 2006)	Balance	11	61.4 ± 9.7 years (48-82)	F	Rotating menu (basal diet including 109 mg Mg), supplemented as follows: i) 0 mg Mg or 200 mg Mg/d ii) 0 mg boron or 3 mg boron/d iii) 0 mg aluminium or 1000 mg aluminium/d	Fixed diet and duplicate diet analysis	24 day test periods	Basal diet Mean Mg daily intake (mg/d): 109 ± 15 Urinary Mg (% diet): 61.2 (n=6) Urinary Ca (% diet): 18.7 (n=6) Faecal Mg (% diet): 31.1 (n=6) Faecal Ca (% diet): 55.6 (n=6) Serum Mg (mmol/l): 0.87 (n=5) Serum Ca (mmol/l): 2.44 (n=5) Erythrocyte Mg (mmol/kg dry wt): 5.30 (n=4) Blood pressure (mm Hg) Diastolic: 69 (n=6) Systolic: 114 (n=6) QRS complex (S) Lead 1: 0.078 (n=4) Lead 2: 0.088 (n=4)	A dietary intake of 109 mg Mg/d resulted in ~zero Mg balance (the authors assume sweat loss of ≤15 mg/d- which was not measured) In those fed no supplemental Mg, boron decreased urinary calcium and increased systolic blood pressure (p=0.05). The width of the QSR complex was decreased by	High

Reference	Study type	n	Age	Sex	Intervention and control or exposure	Dietary intake estimation	Follow up time	Results: effect, mean, SD, RR/OR/HR confidence interval, etc	Overall results	Risk of bias
								Lead 3: 0.085 (n=4) Mg supplemented diet Mean Mg daily intake (mg/d): 340 ± 19 Urinary Mg (% diet): 36.3 (n=5) Urinary Ca (% diet): 23.4 (n=5) Faecal Mg (% diet): 45.4 (n=5) Faecal Ca (% diet): 67.7 (n=5) Serum Mg (mmol/l): 0.91 (n=4) Serum Ca (mmol/l): 2.42 (n=4) Erythrocyte Mg (mmol/kg dry wt): 5.26 (n=4) Blood pressure (mm Hg) Diastolic: 69 (n=5) Systolic: 113 (n=5) QRS complex (S) Lead 1: 0.072 (n=5) Lead 2: 0.080 (n=5) Lead 3: 0.084 (n=5)	boron, but there was no interruption of normal sinus rhythm in any subjects	
Hunt J *et al*, 1998	Balance	21	33 ±7 years (20-42)	F	Rotating menu: i) Lactoovovegetarian with 0 g of meat (367 ± 44 mg/d Mg) ii) Non vegetarian with 184 g of meat, largely beef (260	Fixed diet and duplicate diet analysis	8 week test periods	Plasma Mg Lactoovovegetarian: 0.79 mmol/l Nonvegetarian: 0.81 mmol/l Urinary Mg Lactoovovegetarian: 98 mg/d (27% of diet)	Plasma Mg concentrations tended to be lower with the lactoovovegetarian diet than with the nonvegetarian diet ($p<0.07$). Urinary Mg	High

Reference	Study type	n	Age	Sex	Intervention and control or exposure	Dietary intake estimation	Follow up time	Results: effect, mean, SD, RR/OR/HR confidence interval, etc	Overall results	Risk of bias
					± 29 mg/d Mg)			Nonvegetarian: 89 mg/d (34% of diet) p=0.02 when expressed as % of diet <u>Faecal Mg</u> Lactoovovegetarian: 278 mg/d (76% of diet) Nonvegetarian: 169 mg/d (65% of diet) p=0.0001 for differences expressed as mg/d; p=0.02 for differences expressed as % of diet <u>Apparent absorption:</u> Lactoovovegetarian: 89 mg/d (24% of diet) Nonvegetarian: 91 mg/d (35% of diet) p=0.02 only when expressed as % of diet <u>Balance:</u> Lactoovovegetarian: -9 mg/d (-3% of diet) Nonvegetarian: 2 mg/d (1% of diet)	was only different between the two dietary groups when expressed as a proportion of dietary Mg (i.e. % of diet) Apparent absorption of Mg, expressed as a proportion of dietary Mg, was significantly lower with the lactoovovegetarian diet than with the nonvegetarian diet (p<0.02). Since the lactoovovegetarian diet contained more Mg, the balance did not significantly differ between the two diet periods	
Ivaturi R and Kies C, 1992	Balance	24	N/R	M & F	<u>Study A</u> i) Basal diet (154.0 ± 11.1 mg/d Mg) ii) Basal diet + 60 g sucrose/d (154.0 ± 8.6 mg/d	Fixed diet and dietary analysis	14 day test periods	<u>Study A</u> <u>Adjustment period</u> Faeces (mg/d): 144.8 ± 54.5 Urine (mg/d): 80.6 ± 36.36 Balance (mg/d): -71.4	In Study A, mean faecal losses were higher and balance was less positive on the fructose diet	High

Reference	Study type	n	Age	Sex	Intervention and control or exposure	Dietary intake estimation	Follow up time	Results: effect, mean, SD, RR/OR/HR confidence interval, etc	Overall results	Risk of bias
					Mg) iii) Basal diet + 60 g fructose/d (154.0 ± 10.5 mg/d Mg) <u>Study B</u> i) Basal diet (258 ±18.31 mg/d Mg) ii) Basal diet + 60 g sucrose/d (341 ±13.6 mg/d Mg) iii) Basal diet + 60 g high fructose corn syrup/d (341 ±13.9 mg/d Mg)			<u>Sucrose</u> Faeces (mg/d): 142.0 ± 50.4 Urine (mg/d): 68.2 ± 27.20 Balance (mg/d): -56.2 <u>Fructose</u> Faeces (mg/d): 177.0 ± 49.99 Urine (mg/d): 63.5 ± 20.20 Balance (mg/d): -86.5 <u>Study B</u> <u>Self-selected</u> Faeces (mg/d): 210 ± 39.5 Urine (mg/d): 22.8 ± 7.99 Balance (mg/d): +25.2 <u>Sucrose</u> Faeces (mg/d): 261 ± 28.3 Urine (mg/d): 22.0 ± 7.26 Balance (mg/d): + 58.0 <u>High fructose corn syrup</u> Faeces (mg/d): 262 ± 42.8 Urine (mg/d): 23.2 ± 14.0 Balance (mg/d): +64.8	compared to the sucrose diet ($p<0.05$). In study B, there were no statistical differences between sugars, but Mg balance was higher in both sugar groups compared to the self-selected diet ($p<0.05$) apparently as a result of higher Mg intake	
Knudsen E *et al*, 1996	Balance/ isotope	8	23-27 years	5M & 3F	Rotating menu relatively high in fibre and phytate (9 mmol Mg; 29 g fibre; 1 mmol phytic acid/10 MJ) Mg absorption and retention	Duplicate diet (7 day dietary records at baseline)	21 days	Dietary intake: 10.7 ± 1.5 mmol/d Faecal excretion: 8.3 ± 1.6 mmol/d Apparent absorption: 2.4 ± 1.2 mmol/d	Faecal excretion of Mg was 78% of daily intake Urinary excretion of Mg was 46% of daily intake	High

Reference	Study type	n	Age	Sex	Intervention and control or exposure	Dietary intake estimation	Follow up time	Results: effect, mean, SD, RR/OR/HR confidence interval, etc	Overall results	Risk of bias
					calculated using ^{25}Mg added to one day's diet (50 mg/10 MJ)			Urinary excretion: 4.9 ± 1.2 mmol/d Mg Balance: -2.5 ± 1.0 Absorption (from stable isotopes): 4.9 ± 0.6 mmol/d (46 ± 6%) Endogenous faecal loss (difference between apparent absorption and isotope absorption): 2.7 ± 1.0 mmol/d Baseline serum Mg was 837 ± 78 µmol/l and did not change significantly during study period	Apparent Mg absorption was on average positive (approximately 22% of dietary intake), but Mg balance was negative Intake and fractional absorption of Mg from the high fibre/ phytate study diet was not high enough to compensate for Mg losses	
Lukaski H and Nielsen F, 2002 (Included within Hunt and Johnson, 2006; Witowski *et al*, 2011)	Depletion-Repletion	10	59.7 ± 2.7 years (44.9-71.0)	F	Rotating menu based on western foods. Participants had 35 day equilibration period on the basal diet (112 mg/2000 kcal) plus 200 mg Mg supplement/d (as Mg gluconate). This was followed by a 93 d Mg-depletion period, where the basal diet only was consumed. After Mg depletion, the basal diet was	Duplicate diet	177 days	<u>Means ± SEM:</u> <u>Mg intake (mg/d):</u> Equilibration: 322 ± 17; Depletion: 155 ± 35; Repletion: 360 ± 34 $p<0.05$ for group comparisons <u>Faecal Mg (mg/d):</u> Equilibration: 184 ± 6; Depletion: 119 ± 4; Repletion: 212 ± 5 $p<0.05$ for group comparisons <u>Urinary Mg (mg/d):</u>	Mg balance decreased significantly (net loss of Mg of -42 mg/d) during Mg depletion compared with net retention during equilibration and repletion Mg depletion may be induced by dietary	High

Reference	Study type	n	Age	Sex	Intervention and control or exposure	Dietary intake estimation	Follow up time	Results: effect, mean, SD, RR/OR/HR confidence interval, etc	Overall results	Risk of bias
					supplemented with Mg at 200 mg/d for a further 49 d (Mg repletion)			Equilibration: 107 ± 5; Depletion: 77 ± 8; Repletion: 110 ± 4 p<0.05 for depletion compared to equilibration or repletion <u>Mg Balance (mg/d):</u> Equilibration: +32 ± 6; Depletion: -34 ± 6; Repletion: +38 ± 6 p<0.05 for depletion compared to equilibration or repletion <u>Serum Mg (mmol/l):</u> Equilibration: 0.85 ± 0.02; Depletion: 0.81 ± 0.02; Repletion: 0.86 ± 0.02 p=0.07 for decrease from equilibration to depletion values p=0.06 for increase from depletion to repletion values <u>Erythrocyte Mg (µmol/g Hb):</u> Equilibration: 6.74 ± 0.08; Depletion: 5.91 ± 0.07; Repletion: 6.68 ± 0.08 p<0.05 for equilibration versus depletion <u>Skeletal muscle Mg (mmol/kg dry weight):</u> Equilibration: 51.6 ± 1.3;	Mg restriction in otherwise healthy postmenopausal women. Mg restriction impairs metabolic function during conditions of increased energy expenditure (reflected by a significant increase in peak oxygen uptake, total and cumulative net oxygen uptake, and peak heart rate)	

Reference	Study type	n	Age	Sex	Intervention and control or exposure	Dietary intake estimation	Follow up time	Results: effect, mean, SD, RR/OR/HR confidence interval, etc	Overall results	Risk of bias
								Depletion: 48.1 ± 1.3; Repletion: 53.4 ± 1.2 p<0.05 for depletion compared to equilibration and repletion groups $\underline{V0_2}$ (oxygen consumption) peak, ml/min: Equilibration: 1118 ± 31; Depletion: 1293 ± 43; Repletion: 1128 ±3 1 p<0.05 depletion compared to equilibration or repletion groups $\underline{CNVO_2}$ (cumulative net O2 uptake; ml): Equilibration: 5238 ± 227; Depletion: 6111 ± 232; Repletion: 5319 ± 216 p<0.05 depletion compared to equilibration or repletion groups Heart Rate, bpm: Equilibration: 129 ± 1; Depletion: 136 ± 2; Repletion: 128 ± 1 p<0.05 depletion group compared to equilibration or repletion		

Reference	Study type	n	Age	Sex	Intervention and control or exposure	Dietary intake estimation	Follow up time	Results: effect, mean, SD, RR/OR/HR confidence interval, etc	Overall results	Risk of bias
Milne D and Nielsen F, 2000 (Included within Hunt and Johnson, 2006)	Balance	11	22-40	M	Rotating menu, supplemented with Mg gluconate: i) Starch diet (starch at 20% energy), low Mg (170 mg Mg/2500 kCal) ii) Starch diet (starch at 20% energy), high Mg (370 mg Mg/2500 kCal) iii) Fructose diet (fructose at 20% energy), low Mg (170 mg Mg/2500 kCal) iv) Fructose diet (fructose at 20% energy), high Mg (370 mg Mg/2500 kCal)	Duplicate diet	42 day test periods	<u>Diet Mg (mg/d):</u> Starch-low Mg: 165 Start-high Mg: 370 Fructose-low Mg: 176 Fructose-high Mg: 370 <u>Urine Mg (mg/d):</u> Starch-low Mg: 87 Starch-high Mg: 131 Fructose-low Mg: 89 Fructose-high Mg: 133 Mg effect: P=0.0001 <u>Faecal Mg (mg/d):</u> Starch-low Mg: 89 Starch-high Mg: 209 Fructose-low Mg: 84 Fructose-high Mg: 184 Mg effect: p=0.0001 Fructose effect: p=0.02 <u>Mg balance (mg/d):</u> Starch-low Mg: -12 Starch-high Mg: 30 Fructose-low Mg: 3 Fructose-high Mg: 53 Mg effect: p=0.0001 Fructose effect: p=0.01 <u>Serum Mg (mg/dL):</u> Starch-low Mg: 2.02	Mg balance, faecal, urinary and ultrafilterable Mg were all directly related to Mg intake. Mg balance was independently affected by both Mg and fructose intakes ($p<0.01$). The high fructose diet resulted in higher Mg balance. The men were in negative Mg balance when fed the starch diet containing 165 mg/d Mg. This diet also resulted in the lowest concentrations of ionised and ultrafilterable Mg, indicating 165 mg Mg/d is not adequate Mg treatment did not affect calcium balance, phosphorus	Moderate

Reference	Study type	n	Age	Sex	Intervention and control or exposure	Dietary intake estimation	Follow up time	Results: effect, mean, SD, RR/OR/HR confidence interval, etc	Overall results	Risk of bias
								Starch-high Mg: 2.03 Fructose-low Mg: 2.06 fructose-high Mg: 2.07 <u>Ionized Mg (mg/dL):</u> Starch-low Mg: 1.34 Starch-high Mg: 1.36 Fructose-low Mg: 1.36 Fructose-high Mg: 1.39 <u>Ultrafilterable Mg</u> Starch-low Mg: 1.40 Starch-high Mg: 1.45 Fructose-low Mg: 1.45 Fructose-high Mg: 1.48 Mg effect: p=0.02 Fructose effect: p=0.04	balance, alkaline phosphatase, osteocalcin or parathyroid hormone	
Nielsen F and Milne D, 2003 (Included within Hunt and Johnson, 2006; Witowski *et al*, 2011)	Balance	19	47-78 years	F	Rotating menu: i) Low Mg/low Cu (99 mg/2000 kcal Mg, 1 mg/2000 kcal Cu) ii) Low Mg/high Cu (99 mg/2000 kcal Mg, 3 mg/2000 kcal Cu) iii) High Mg/low Cu (399 mg/2000 kcal Mg, 1 mg/2000 kcal Cu) iv) High Mg/high Cu (399 mg/2000 kcal Mg, 3	Duplicate diet	81 day test periods	Mg balance during last 24 d of each 81 d dietary phase (regardless of sequence of diets): <u>Diet Mg, (mg/d):</u> Low Mg/low Cu: 118 Low Mg/high Cu: 131 High Mg/low Cu:408 High Mg/high Cu: 414 <u>Faecal Mg (mg/d):</u> Low Mg/low Cu: 60 Low Mg/high Cu: 60	Plasma ionized Mg was significantly lower during the low Mg phase. Serum Mg was lower when the Mg restriction occurred first Several variables indicated that low dietary Cu affected the response to Mg	High

Reference	Study type	n	Age	Sex	Intervention and control or exposure	Dietary intake estimation	Follow up time	Results: effect, mean, SD, RR/OR/HR confidence interval, etc	Overall results	Risk of bias
					mg/2000 kcal Cu) The high Mg diet was supplemented with 300 mg Mg/d as magnesium gluconate			High Mg/low Cu: 181 High Mg/high Cu:182 Mg effect p=0.01 <u>Urine Mg (mg/d):</u> Low Mg/low Cu:62 Low Mg/high Cu: 75 High Mg/low Cu:130 High Mg/high Cu:144 Mg effect p=0.0001 <u>Mg balance (mg/d):</u> Low Mg/low Cu: -5 Low Mg/high Cu: -3 High Mg/low Cu: +97 High Mg/high Cu: +88 Mg effect p=0.0001 <u>Serum total Mg (mM/l):</u> Low Mg/low Cu: 0.83 Low Mg/high Cu:0.85 High Mg/low Cu:0.88 High Mg/high Cu:0.86 <u>Plasma ionized Mg (mM/l):</u> Low Mg/low Cu: 0.56 Low Mg/high Cu:0.56 High Mg/low Cu:0.58 High Mg/high Cu:0.58 Mg effect p=0.008	deprivation: Mg deprivation elevated SOD at higher intakes of dietary Cu, when dietary Cu was lower, Mg deprivation had no effect on SOD activity; erythrocyte Mg was lower when dietary Cu was low; when Mg intake was low, serum Cu was lower than in those fed low Cu; when Mg intake was high, low dietary Cu did not affect serum Cu. Dietary copper intake did not significantly affect Mg balance Total cholesterol and LDL cholesterol were lower during Mg restriction than during Mg supplementation when Mg restriction occurred first (p for	

Reference	Study type	n	Age	Sex	Intervention and control or exposure	Dietary intake estimation	Follow up time	Results: effect, mean, SD, RR/OR/HR confidence interval, etc	Overall results	Risk of bias
									Mg x sequence x Cu =0.0003 and 0.001 for total cholesterol and LDL cholesterol respectively) Dietary intake of about 100 mg/2000 kcal Mg leads to depleted body stores of Mg and changes in oxidative and lipid metabolism	
Nielsen F and Milne D, 2004a	Balance	21	64.9 ± 6.7 years (50-76)	F	Rotating menu low in copper (2 mg) and zinc (9 mg/2000 kcal), and supplemented with Mg to provide 300 mg daily (180 mg as Mg gluconate). After an initial 10 d equilibration phase (basal diet), 90 d experimental diet with low copper (1 mg/2000 kcal)/low zinc (3 mg/2000 kcal) (n=9) or high copper (3 mg/2000 kcal)/low zinc diets (3 mg/ 2000 kcal) (n=12),	Duplicate diet	200 days (total)	<u>Dietary Mg (mmol/d):</u> LC/LZ: 13.5; LC/HZ: 12.88; HC/LZ: 13.74; HC/HZ: 12.76 <u>Faecal Mg (mmol/d) (pooled SDs, not extracted):</u> LC/LZ: 8.19; LC/HZ: 8.44; HC/LZ: 8.64; HC/HZ: 8.48 <u>Faecal Mg (% intake):</u> LC/LZ: 60.9; LC/HZ: 65.5; HC/LZ: 63.0; HC/HZ: 66.7 (p=0.05 for zinc effect) <u>Urine Mg (mmol/d):</u> LC/LZ: 4.32; LC/HZ: 4.40; HC/LZ: 4.03; HC/HZ: 4.03	The percentages of dietary Mg which appeared in the faeces and urine were significantly higher in the high dietary zinc periods than the low dietary zinc periods. This resulted in a significantly decreased Mg balance during high zinc dietary phases	High

Reference	Study type	n	Age	Sex	Intervention and control or exposure	Dietary intake estimation	Follow up time	Results: effect, mean, SD, RR/OR/HR confidence interval, etc	Overall results	Risk of bias
					2nd 10 d equilibration phase, followed by 90 d experimental diet with low copper (1 mg/2000 kcal) /high zinc (53mg/2000kcal) (n=9) or high copper (3 mg/2000 kcal) /high zinc (53 mg/2000 kcal) (n=12)			Urine Mg (% intake): LC/LZ: 32.1; LC/HZ: 34.3; HC/LZ: 29.4; HC/HZ: 31.6 (p<0.005 for zinc effect) Mg Balance (mmol/d): LC/LZ: +0.95; LC/HZ: +0.04; HC/LZ: +1.07; HC/HZ: +0.23 (p=0.004 for zinc effect) Plasma ionised Mg concentrations (mmol/l): Eq1: 0.56; LC/LZ: 0.56; LC/HZ: 0.55; Eq2: 0.53; HC/LZ: 0.54; HC/HZ: 0.54	Mg balance and plasma ionised Mg were unaffected by changes in copper High dietary intake of zinc had an apparently unfavourable effect on Mg balance by increasing faecal and urinary Mg excretion	
Nielsen F, 2004b (Included within Hunt and Johnson, 2006; Witowski *et al*, 2011)	Balance	13	50-78 years	F	Rotating menu supplemented with magnesium gluconate and sodium borate capsules as follows: i) Low Mg-low Boron (118 mg/d Mg, 0.25 mg/d B) ii) Low Mg-high Boron (118 mg/d Mg, 3.25 mg/d B) iii) High Mg-low Boron (318 mg/d Mg, 0.25 mg/d B) iv) High Mg-high Boron (318 mg/d Mg, 3.25 mg/d B)	Duplicate diet	42 day test periods	Last 24 d of each 42 d dietary phase: Diet, mg/d: Low Mg/low B (n=13): 116 Low Mg/high B (n=12): 119 High Mg/low B (n=13): 321 High Mg/high B (n=13): 317 Faecal Mg, mg/d (% intake): Low Mg/low B: 60 (52.2) Low Mg/high B: 51 (43.3) High Mg/low B: 177 (55.1) High Mg/high B: 170 (53.7) Mg effect p=0.0001 (p=0.08)	An intake of ~318 mg/d of Mg resulted in a positive Mg balance and an intake of ~118 mg/d of Mg resulted in a negative balance Serum Mg was not significantly affected by changes in Mg dietary intake	High

Reference	Study type	n	Age	Sex	Intervention and control or exposure	Dietary intake estimation	Follow up time	Results: effect, mean, SD, RR/OR/HR confidence interval, etc	Overall results	Risk of bias
								Urinary Mg, mg/d (% intake): Low Mg/low B: 74 (63.4) Low Mg/high B: 74 (62.1) High Mg/low B: 141 (43.9) High Mg/high B: 137 (43.0) Mg effect: p=0.0001 (p=0.0001) Mg balance, mg/d: Low Mg/low B: -18 Low Mg/high B: -6 High Mg/low B: +3 High Mg/high B: +10 Mg effect: p=0.003	Mg deficiency significantly increased calcium balance (p=0.45), but had no effect on phosphorus, zinc or manganese balance. Mg deficiency increased serum 25-hydroxycholecalcifer ol (p=0.01) but did not affect serum calcitonin, parathyroid hormone, ostecalcin, or alkaline phosphatase Mg deficiency decreased serum total cholesterol (p=0.02) An intake of ~118 mg/d of Mg was not aqeduate in post-menopausal women. Furthermore, one woman exhibited heart ventricular	

Reference	Study type	n	Age	Sex	Intervention and control or exposure	Dietary intake estimation	Follow up time	Results: effect, mean, SD, RR/OR/HR confidence interval, etc	Overall results	Risk of bias
									ectopy after consuming the low Mg diet, which disappeared upon Mg supplementation Changes in dietary boron had no significant effect on Mg balance or the response to Mg deprivation	
Nielsen F *et al*, 2007a (Included within Witowski *et al*, 2011)	Balance	11	62.9 ± 7.3 years (49-71)	F	Rotating menu: i) Basal diet (supplying a mean of 107 mg Mg/d) supplemented with 220 mg/d of Mg as magnesium gluconate ii) Basal diet supplemented with placebo (lactose)	Duplicate diet	72 day test periods	<u>Dietary Mg (mmol/d):</u> Mg deficient: 4.40; Mg supplemented: 13.46 <u>Faecal Mg, mmol/d; (% intake):</u> Mg deficient: 2.47 (56.2); Mg supplemented: 6.95 (51.7) (p=0.0001 and p=0.005 for diet effect) <u>Urine Mg, mmol/d (% intake):</u> Mg deficient: 2.14 (49.3); Mg supplemented: 4.28 (31.7) (p=0.0001 for diet effect) <u>Mg balance, mmol/d:</u> Mg deficient: -0.21; Mg supplemented: +2.22 (p=0.0001	Erythrocyte Mg was significantly decreased during Mg deprivation, as was serum Mg when deprivation occurred first Mg balance was positive when the diet provided ~327 mg Mg/d, but negative when the diet provided ~107 mg Mg/d (despite a	Low

Reference	Study type	n	Age	Sex	Intervention and control or exposure	Dietary intake estimation	Follow up time	Results: effect, mean, SD, RR/OR/HR confidence interval, etc	Overall results	Risk of bias
								for diet effect) Serum Mg (mmol/l): Mg deficient: 0.86; Mg supplemented: 0.88 (p=0.04 for diet effect) RBC Mg (nmol/mg protein) Mg deficient: 2.51; Mg supplemented: 2.67 (p=0.05 for diet effect)	significant decrease in urinary Mg excretion) Calcium balance was significantly higher during the Mg deficient phase (p=0.009). There was little effect on overall phosphorus balance, serum indicators of calcium metabolism, 25-hydroxycholecalciferol, or alkaline phosphatase 3 subjects showed changes in the low Mg phase which required supplementation of Mg (increases in ventricular discharges, BP, poor wound healing)	

Reference	Study type	n	Age	Sex	Intervention and control or exposure	Dietary intake estimation	Follow up time	Results: effect, mean, SD, RR/OR/HR confidence interval, etc	Overall results	Risk of bias
Nielsen F *et al*, 2007b (Included within Witowski *et al*, 2011)	Balance	13	61.8 ± 8.2 years (47-75)	F	Rotating menu based on western foods (101 mg Mg/2000 kcal) for 78 days, then basal diet supplemented with 200 mg Mg/d as Mg gluconate for 58 days	Duplicate diet	136 days (total)	Ingested Mg (mmol/d): Mg-low: 4.44; Mg-high: 13.13 p=0.0001 Faecal Mg (mmol/d); (% intake): Mg-low: 2.06 (46.2); Mg-high: 6.67 (50.7) p=0.0001 Urinary Mg (mmol/d); (% intake): Mg-low: 2.51 (57.4) ; Mg-high: 4.44 (34.5) p=0.0001 Balance Mg (mmol/d): Mg-low: -0.12; Mg-high: 1.98 Serum Mg (mmol/l): Mg-low: 0.79; Mg-high: 0.74 p=0.0001 RBC membrane Mg (nmol/mg protein): Mg-low: 3.00; Mg-high: 3.25 p=0.005 Serum total cholesterol (mmol/l): Mg-low: 6.10; Mg-high: 6.67 p=0.0003	Total cholesterol was significantly decreased during the Mg-low diet period. Changes to other blood lipids were not significant The IVGTT test during the Mg-low diet period showed that serum glucose increased to a higher concentration and remained high until the end of the test compared to the test performed during the Mg-high period. The area under the curve for glucose was significantly greater ($p<0.006$) during the Mg-low diet period. Glucagon also decreased ($p<0.02$). There was no change in the insulin reponse	Moderate

Reference	Study type	n	Age	Sex	Intervention and control or exposure	Dietary intake estimation	Follow up time	Results: effect, mean, SD, RR/OR/HR confidence interval, etc	Overall results	Risk of bias
									5 subjects presented with heart arrhythmias during the Mg-low diet period. The arrhythmias which responded quickly to Mg supplementation were atrial flutter and fibrillation. Blood pressure was not significantly affected Urinary Mg excretion significantly decreased when the diet was Mg-low, but balance was negative (although not significantly different from zero). An intake of ~101 mg Mg/2000 kcal may be inadequate for post-menopausal women and may induce heart arrhythmias and	

Reference	Study type	n	Age	Sex	Intervention and control or exposure	Dietary intake estimation	Follow up time	Results: effect, mean, SD, RR/OR/HR confidence interval, etc	Overall results	Risk of bias
									impair glucose homeostasis	
Rude R *et al*, 1991	Depletion	24	N/R	M & F	Volunteers fed a liquid low Mg diet (<1 mEq/d) for 3 weeks Erythrocyte and serum Mg status was determined	Not reported	3 weeks	Serum Mg (mg/dL) Pre-diet: 1.9 ± 0.02; post-diet: 1.5 ± 0.05 (p<0.001) Mg tolerance testing- load retention (%): Pre-diet: 11 ± 4; post-diet: 62 ± 4 (P<0.001) RBC Mg (µM) Pre-diet: 208 ± 9.8; week 1: 174 ± 12.5; week 2: 155 ± 7.2; post-diet (week 3): 162 ± 9.3 (p<0.001)	RBC and serum Mg concentrations reflect developing Mg deficiency A positive correlation was reported between serum and RBC Mg levels (r=0.54, p<0.001)	High
Sabatier M *et al*, 2002	Isotope	10	25-45	F	Magnesium rich mineral water (110 mg Mg/l): i) 500 ml water consumed alone, followed by a test meal ~3 hours later containing ~17 mg Mg (breakfast of 56g toast, 10g butter and 30g jam) ii) 500 ml water consumed concurrently with the test meal	Duplicate diet	Complete faecal collection	Mean ± SD: Mg absorption (%): Without meal: 45.7 ± 4.6 (range 40.2-55.5) With meal: 52.3 ± 3.9 (range 46.2-60.2) p<0.05 compared to without meal Mg retention (%): Without meal: 37.4 ± 4.0 (range 33.1-47.0) With meal: 41.5 ± 4.2 (range 35.2-50.6) p<0.05 compared to	Total dietary intake of Mg during test period, including that from mineral water and stable isotopes was 329 ± 54 mg Mg absorption from water consumed with test meal was higher than from water alone, with an increase of 6.6 ± 3.2	High

Reference	Study type	n	Age	Sex	Intervention and control or exposure	Dietary intake estimation	Follow up time	Results: effect, mean, SD, RR/OR/HR confidence interval, etc	Overall results	Risk of bias
					Mg absorption and retention was determined using ^{25}Mg and ^{26}Mg			without meal	% (p=0.0001) relative increase of 14.4% There was also a significant increase in urinary excretion of labelled Mg from 18.2% to 20.6% of the absorbed dose (p=0.019) when water was taken with the meal Absolute increase in Mg retention when mineral water was consumed with a meal was 4.1 ± 2.7% (p=0.0004) or a relative increase of 11% Mg absorption and retention were enhanced when the water was consumed with a meal	

Reference	Study type	n	Age	Sex	Intervention and control or exposure	Dietary intake estimation	Follow up time	Results: effect, mean, SD, RR/OR/HR confidence interval, etc	Overall results	Risk of bias
Sabatier M *et al*, 2011	Isotope	11	24.5 ± 1.7 years (21-27)	M	Magnesium rich mineral water: i) 126 mg Mg consumed as a bolus dose (2 x 750 ml water/d) ii) 126 mg Mg consumed in multiple doses (7 x 212 ml water/d) Mg absorption and retention was determined using ^{25}Mg and ^{26}Mg	Unclear	Complete faecal collection	<u>Mg absorption (mean ± SD)</u> 2 servings/d: 32.4 ± 8.1 % 7 servings/d: 50.7 ± 2.7% Significant increase of 18.3 % (95% CI 10.1, 26.5), p=0.0007, or a relative increase of 56.4% <u>Mg retention (mean ± SD)</u> 2 servings/d: 29.0 ± 7.5 % 7 servings/d: 47.5 ± 12.9 % The absolute increase in Mg retention was 18.5% (95% CI 10.0, 26.9), p=0.0008, or a relative increase of 63.8%	Regular Mg-rich mineral water consumption throughout the day may increase Mg bioavailability	High
Turnlund J *et al*, 1992	Balance	8	21-30 years	F	Study divided into 6 metabolic periods (MPs): MP1-adaptation period: food diet for 4 d followed by formula diet containing 2 mg Vit B6/d for 3 d MP2: B6 depletion period: formula diet containing <0.05 mg/d vitamin B6 for up to 28 d (until evidence of Vit B6 depletion observed) After MP2, women randomly assigned to 3 d	Analysis of daily diets	84-98 d	<u>Mg balance (mean ± SE) mg/d:</u> <u>Animal protein diet:</u> B6 depletion diet (MP2): -49 ± 4 B6 repletion phases (MP3-6): +14 ± 11 ($p<0.05$ compared to depletion phase) <u>Plant protein diet:</u> B6 depletion diet (MP2): -69 ± 4 B6 repletion phases (MP3-6): +10 ± 28 ($p<0.05$ compared to	Serum Mg was not influenced by diet or dietary vitamin B6 intake Mg balance was significantly lower during vitamin B6 depletion dietary phase than with either repletion diet (animal or plant protein-based)	High

Reference	Study type	n	Age	Sex	Intervention and control or exposure	Dietary intake estimation	Follow up time	Results: effect, mean, SD, RR/OR/HR confidence interval, etc	Overall results	Risk of bias
					rotating menu of either animal-protein or plant-protein diets (both diets contained 0.5 mg/d Vit B6). MP3: 14 d on diet without added B6 MP4: 14 d on diet supplemented with 0.5 mg/d B6 MP5: 21 d on diet supplemented with 1.0 mg/d B6 MP6: 14 d on diet supplemented with 1.5 mg/d B6 Formula diet contained 327 mg/d Mg Animal protein diet contained average 294 mg/d Mg Plant protein diet contained average 520 mg/d Mg			depletion phase) <u>Urinary Mg excretion (mean ± SE) mg/d:</u> B6 depletion (MP2 <0.05 mg/d B6): 176 ± 9 p<0.05 compared to repletion phases MP3 (0.5 mg/d B6): animal protein: 111; plant protein: 128 MP4 (1.0 mg/d B6): animal protein: 115; plant protein: 143 MP5 (1.5 mg/d B6): animal protein: 111; plant protein: 145 MP6 (2mg /d B6): animal protein: 108; plant protein: 144	Urinary Mg was significantly higher in the vitamin B6 depletion diet than with either the animal-or plant-protein diet, even though the plant protein diet contained more Mg than the depletion diet The results suggest that vitamin B6 deficiency may have a deleterious effect on Mg metabolism	
Walti M *et al*, 2003	Isotope	10	71.6 ± 5.5 years	7M & 3F	Test meal of a low phytate wheat bread-roll and 300 g isotopically labelled water (10 mg ^{26}Mg). Total Mg	Not reported	Complete faecal collection	<u>Mean ± SD:</u> Apparent Mg absorption (% administered dose): 57.6 ± 8.5	Mean apparent Mg absorption was 57.6 % of administered dose (there were no	Low

Reference	Study type	n	Age	Sex	Intervention and control or exposure	Dietary intake estimation	Follow up time	Results: effect, mean, SD, RR/OR/HR confidence interval, etc	Overall results	Risk of bias
					content of the test meal was 23.6 ± 1.0 mg			Urinary Mg (% absorbed dose): 11.7 ± 3.8 Mg retention (% administered dose): 51.4 ± 6.1 Total urinary Mg in 6-d pool (mg): 482.8 ± 184.3 Mean daily Mg excretion (mg): 80.5 ± 30.7	significant differences between diabetic and control subjects)	
Witowski M *et al*, 2011	SR	27 (a)	8-89 years	M & F	Magnesium supplementation or depletion studies which assess a change in biomarker status (RCTs, CTs, before-after studies)	Not reported	1 day – 52 weeks	Significant responses to magnesium intake were found for: serum/ plasma concentrations (weighted mean difference [WMD]: 0.03 mmol/l, 95% CI 0.01, 0.06, I^2 96%, p<0.02); erythrocyte magnesium concentration (WMD: 0.16 mmol/l, 95% CI 0.09, 0.22, I^2 85%, p<0.0001); and urinary magnesium (WMD: 1.82 mmol/24 h, 95% CI 1.29, 2.36, I^2 93%, p<0.00001)	Serum/ plasma, erythrocyte and urinary Mg excretion appear to be useful biomarkers of Mg status, although more high quality studies are needed in this area	High

(a): The number of studies included is reported, rather than the number of participants. Six studies included in Hunt and Johnson, 2006 met our inclusion criteria (as cross-referenced in **Table 4** here). Seven studies included in Witowski et al, 2011 met our inclusion criteria (as cross-referenced in **Tables 2-4**)

Table 5. Breast milk concentration

Reference	n women (samples)	Country	Maternal intake	Stage of lactation	Mg level (mg/l) mean ±SD median	Range (mg/l)	Risk of bias
Bocca B *et al*, 2000	60 (60 samples)	Italy	Not reported	Not reported	Mean ± SD: 23.0 ± 0.51 Median: 4.11		High
Dorea J, 2000	54	Brazil	Not reported	28-380 days		24-28	High
Doybak A *et al*, 1999	35	Turkey	Not reported	1-4 months (28-123 days)	Mean ± SD: 1st month: 28 ± 7 4th month: 31 ± 8		High
Friel J *et al*, 1999	19 (136 samples)	Canada	Not reported	2/3 days-3 months	Mean ± SD: week 1: 30.41 ± 4.74 week 2: 26.69 ± 3.98 week 3: 26.25 ± 4.40 week 4: 26.73 ± 4.71 week 5: 28.33 ± 5.59 week 6: 29.20 ± 5.02 week 7: 31.47 ± 5.76 week 8: 33.20 ± 5.24 week 12: 34.58 ± 6.02	26-35	High
Holt C, 1993	4 (28 samples)	UK	Not reported	1 day-4 months	Mean ± SD: Total: 33.05 ± 3.4 Ultrafiltrate: 27.7 ± 3.89 Colloidal: 5.10 ± 1.70		High

Reference	n women (samples)	Country	Maternal intake	Stage of lactation	Mg level (mg/l) mean ±SD median	Range (mg/l)	Risk of bias
Hunt C *et al*, 2005	45	USA	Not reported	1-4 months	Mean ± SD: Month 1: 28.6 ± 2.2 Month 4: 33.0 ± 2.2		High
Parr R *et al*, 1991	81 71 15 65 29 69	Guatemala Hungary Nigeria Philippines Sweden Zaire	Not reported	~ 3 months	Median ± SD: Guatemala: 34.1 ± 0.9 Hungary: 32.6 ± 0.7 Nigeria: 29.0 ± 2.6 Philippines: 29.7 ± 0.7 Sweden: 34.2 ± 2.3 Zaire: 37.8 ± 0.9		High
Rakicioglu N *et al*, 2006	21	Turkey	During Ramadan: 194.2 ± 71.8 After Ramadan: 219.0 ± 81.2	2-5 months	Mean ± SD: During Ramadan: 29 ± 5 After Ramadan: 33 ± 5		High
Sievers E *et al*, 2000	14 infants	Germany	Not reported	Infant age median 3.6 weeks (range 2.6-4.7)	Median and range: 28.6	21.2-44	High
Sinchai W *et al*, 1995	112	Thailand	Not reported	6 weeks		Mean range: 24.1-24.8	High

Reference	n women (samples)	Country	Maternal intake	Stage of lactation	Mg level (mg/l) mean ±SD median	Range (mg/l)	Risk of bias
Tanzer F and Sunel S, 1991	20	Turkey	405 mg	Up to 26 weeks	Mean (SE): 3 weeks: 41.6 (1.5) 26 weeks: 46.7 (1.5)		High
Vitolo M *et al*, 2004	90	Brazil	Not reported	30-90 days	Average values: Low socio-economic adolescents: 25.76 ± 4.37 Low socio-economic adults: 28.19 ± 5.59 High socio-economic adults: 26.98 ± 5.59		High
Yamawaki N *et al*, 2005	1170 samples	Japan	Not reported	1-365 days	Mean Total: 27 ± 9 By season Summer: 26 ± 9 Winter: 27 ± 9 By stage of lactation: Day 1-5: 32 ± 5 Day 6-10: 30 ± 9 Day 11-20: 29 ± 6 Day 21-89: 25 ± 7 Day 90-180: 27 ± 11 Day 181-365: 33 ± 7		High

(a): Breast milk data from Dengel *et al*, 1994 were included in **Table 4**

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SCIENTIFIC REPORT submitted to EFSA

Literature search and review related to specific preparatory work in the establishment of Dietary Reference Values

Preparation of an evidence report identifying health outcomes upon which Dietary Reference Values could potentially be based for magnesium, potassium and fluoride [3]

Potassium

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[3] **(Question No EFSA-Q-2010-01547). Accepted for publication on 14 May 2012.**

Abstract

The objective of this systematic search and review was to identify the scientific data from January 1990 to September 2011 upon which Dietary Reference Values (DRVs) may potentially be based for potassium.

Articles were identified using Medline, EMBASE (both on Ovid SP) and the Cochrane Library CENTRAL databases. Duplicate references were removed and additional studies identified by checking reference lists of pertinent reviews, included studies and current reports on DRVs. A total of 2583 articles were screened on the basis of title and abstract, resulting in 235 articles retrieved for full-text assessment, and the final inclusion of 44 studies (46 articles) for this review.

The majority of studies included investigated metabolism and nutrient interactions (12). The remaining studies reported blood pressure (9), potassium content in breast milk (9), bone health (6), aldosterone and renin (5), diabetes (1), resting metabolic rate (1) and sleep (1). Study designs included randomised control trials, non-randomised controlled trials, balance, cohort and cross-sectional studies. Two studies were assessed as being at moderate risk of bias and 42 at high risk of bias. Overall, there appeared to be insufficient high quality studies.

Summary

This systematic search and review was carried out preparatory to work by EFSA to establish Dietary Reference Values (DRVs) for potassium, magnesium and fluoride, Lot 3 from the open call for tender CFT/EFSA/NDA/03. This report summarises the findings on potassium.

The literature was comprehensively searched from January 1990 to September 2011 for studies in the English language. The search focused on primary research in humans concerning maintenance of functional competence and the prevention of clinical deficiency and chronic disease upon which DRVs may be based. Only studies reporting a quantitative relationship between i) intake and status; ii) intake and health; or iii) status and health were included (with the exception of studies reporting potassium concentration in breast milk).

Articles were identified using Medline, EMBASE (both on Ovid SP) and the Cochrane Library CENTRAL databases. Complex search strategies using index and text terms, truncating and Boolean operators were developed and refined for each database. The search results were combined and imported into Endnote® (version X4, Thomson Reuters, New York) and duplicate references were removed, resulting in 2567 references to screen. A further 16 articles were identified by checking reference lists of pertinent reviews, included studies and current reports on DRVs. In total, 2583 articles were screened on the basis of title and abstract, resulting in 235 articles being retrieved for full text assessment, and the final inclusion of 46 articles, representing 44 studies, for this review which satisfied all inclusion criteria. For each study, data on design, methodology, results and validity were fully extracted into a Microsoft Excel® (Microsoft Corp, Seattle) database, and the key data summarised.

Two studies assessed as being at moderate risk of bias and 44 at high risk of bias were included.

The studies included: 22 randomised controlled trials; 8 non-randomised controlled trials; 4 balance studies; 1 cohort study; and 9 cross-sectional studies. The majority of studies included were investigating metabolism and nutrient interactions (12). The remaining studies reported blood pressure (9), potassium content in breast milk (9), bone health (6), aldosterone and renin (5), diabetes (1), resting metabolic rate (RMR) (1) and sleep (1).

Health endpoints determined were mainly related to metabolism and nutrient interactions, blood pressure, and bone health. There were limited data on diabetes, RMR and sleep. The status markers employed were serum potassium concentration and urinary potassium excretion, both of which appear to reflect short term intake. Study populations mainly included adults, infants and lactating mothers. There were three studies on adolescents but no studies on children. Overall, evidence published between January 1990 and September 2011, regarding potassium in relation to the setting of DRVs appeared to be of poor quality.

Key words: potassium, systematic review, Dietary Reference Values (DRVs), dietary requirements, health outcomes, biomarkers, status, bioavailability

Introduction

This report focused on identifying information to inform the setting of Dietary Reference Values (DRVs) for potassium, which is an essential element, required for normal cell function and involved in fluid, acid and electrolyte balance. Additionally, potassium is a co-factor for pancreatic insulin secretion, creatine phosphorylation, carbohydrate metabolism, and protein synthesis (EVM, 2003). Previously the SCF (1993) set a population reference intake (PRI) for adults of 3100 mg/day.

This review only reports on potassium forms present naturally in foods or those approved by the EC (EC Directive 2002/46/EC; EC Regulation No 1925/2006) in foods or food supplements (potassium bicarbonate, potassium carbonate, potassium chloride, potassium citrate, potassium fluoride, potassium gluconate, potassium glycerophosphate, potassium lactate, potassium hydroxide, potassium salts of orthophosphoric acid, magnesium potassium citrate, potassium iodide, iodate, potassium L-pidolate, potassium malate, and potassium molybdate). Potassium is found in most foods except highly refined food such as pure sugars, fats and oils. Fruits and vegetables are a good source of potassium. Urinary potassium may be a good marker of short term intake as the majority of potassium is excreted via the urine. Total body potassium can be assessed by ^{42}K dilution, whole body counting, or by direct measurement in tissues such as muscle biopsies, but these are quite invasive and therefore not commonly used in nutrition research. Plasma and serum potassium concentrations are tightly regulated and the majority of potassium is intracellular, which limits their usefulness as markers of status (EVM, 2003; IOM, 2006).

Specific objectives and methodology

The purpose of this work was to collate the scientific data from which Dietary Reference Values for potassium may be derived, building on existing advice of the Scientific Committee for Food Dietary Reference Values report of 1993.

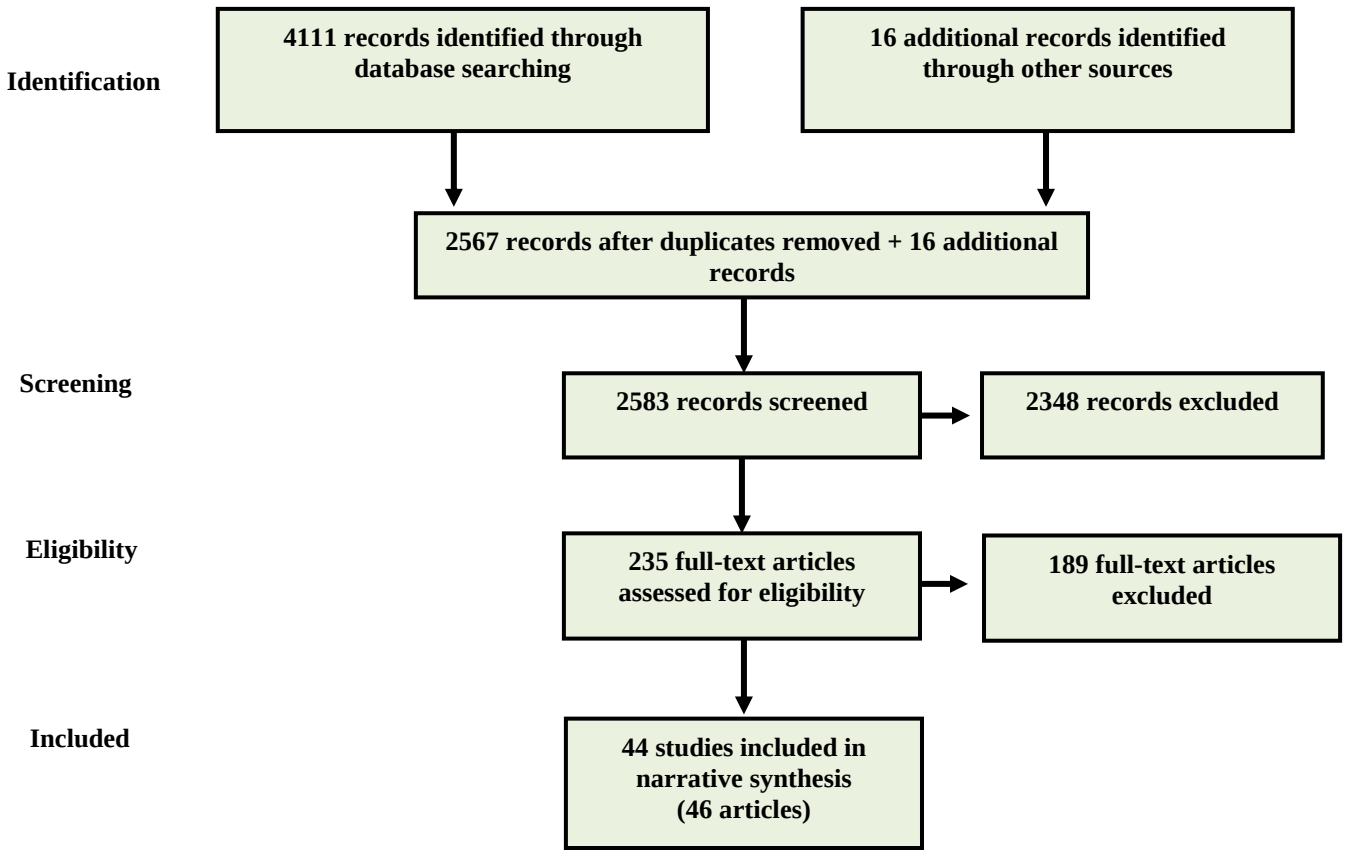
In September 2011, the electronic searches were run following rigorous development and optimisation of the complex search strategy (which included indexing and text terms, truncation and Boolean operators). Potassium specific search strategies are detailed in **Appendix A: Potassium.**

The methods for article screening, full text assessment, data extraction and validity assessment were as described in the Materials and Methods section above. Specific to potassium, studies investigating blood pressure which were shorter than four weeks in duration were excluded.

Results

A total of 4111 records were identified through database searching, duplicate references (1544) were removed, resulting in 2567 references to screen. A further 16 articles were identified by checking reference lists of pertinent reviews, included studies and current reports on DRVs. In total, 2583 articles were screened on the basis of title and abstract, resulting in 235 articles retrieved for full text assessment, and the final inclusion of 44 studies (46 articles) for this review which satisfied all inclusion criteria. These results are summarised in the PRISMA flow chart (**Figure 1**; Moher *et al*, 2009):

Figure 1. PRISMA flow chart



A total of 44 studies were included: 22 randomised controlled trials (RCTs); 8 non-randomised controlled trials (CTs); 4 balance studies; 1 cohort study; and 9 cross-sectional studies. The studies included have been grouped under the following headings: metabolism and nutrient interactions; blood pressure; breast milk potassium concentration; bone health; aldosterone and renin; resting metabolic rate; and sleep. Thirty two studies were carried out in adults: 6 in females; 6 in males; and 20 in mixed gender populations. Three studies were carried out in adolescents: 1 in females; and 2 in mixed gender populations. Nine studies reported potassium breast milk concentrations. The included studies are summarised by primary endpoint, study type and population in **Table 1**. The study methodology, results and quality are described in detail in the sections below and summarised by endpoint in **Tables 2-9**.

Table 1. Summary of studies included [(a)]

Endpoint	Study type	Population
Metabolism and nutrient interactions (12)	RCT (3), CT (6), balance (3),	Adult, male (3) Adult, mixed gender (8) Adolescent, females (1)
Blood pressure (9)	RCT	Adult, female (1) Adult, mixed gender (7) Adolescent, mixed gender (1)
Aldosterone and renin (5)	RCT (4), CT (1)	Adult, males (1) Adult, females (1) Adult, mixed gender (3)
Bone Health (6)	RCT (5), balance (1)	Adult, females (4) Adult, mixed gender (1) Adolescent, mixed gender (1)
Diabetes (1)	cohort	Adult, mixed gender
Resting metabolic rate (1)	CT	Adult, males
Sleep (1)	RCT	Adult, males
Breast milk concentration (9)	Cross-sectional	Infants and lactating females

(a): Number of studies included for each endpoint, study type and population is given in brackets

Validity

Validity was assessed for each study; no studies with low risk of bias, two with moderate risk of bias, and 42 with a high risk of bias were included. Randomised controlled trials were assessed as being at moderate risk of bias when allocation concealment was unclear or compliance was not reported, and at high risk of bias when studies were not blinded, or background diet was not reported, or validity for analytical procedures were not reported. The included CTs were assessed as being at high risk of bias as they were not randomised, or blinded, or did not adequately report dropouts, or carry out a dose check. Balance studies were assessed as being at high risk of bias as they did not adequately deal with confounding factors,

did not adequately measure background exposure, or did not adequately report dropouts. The cohort study was assessed as being at high risk of bias as the study did not adequately deal with confounding factors and did not fully report outcomes. All cross-sectional studies (breast milk concentration endpoint) were assessed as being at high risk because they did not adequately report maternal potassium intake, or did not report validity of potassium analysis, or the study group was not from a representative population.

METABOLISM AND NUTRIENT INTERACTIONS (TABLE 2)

Metabolism

Studies in this section focused mainly on metabolism and nutrient interactions, and also reported on differences in both end-points that were related to race. Of the 12 studies included, three were RCTs, six were CTs, and three were balance. All studies were assessed as being at high risk of bias. Twelve of the studies were carried out in adults, three in males, one in females and eight in mixed gender populations. One of the studies was carried out in adolescent females.

Schmidlin *et al* (1999) carried out a study in 16 adults (14 men, 2 women) aged 25-50 years, initially giving a basal diet with low salt (NaCl) and low potassium (15 mmol Na and 1170 mg potassium/70 kg body weight/day).This was followed by a high salt, low potassium (250 mmol/70 kg/day NaCl and 1170 mg potassium/70 kg body weight/ day) and high salt, high potassium diet (high NaCl diet plus 3056 mg potassium/70 kg body weight/day potassium bicarbonate). Ten subjects were reported to be salt-sensitive and six were salt-resistant. Potassium bicarbonate increased serum potassium in both salt-resistant and salt-sensitive individuals. Urinary potassium excretion increased following high potassium diet in both salt-sensitive and salt-resistant individuals. Dietary potassium supplementation reversed the NaCl induced increases in renal vascular resistance (RVR) and filtration fraction (FF) observed in salt -sensitive subjects; both salt-sensitive and salt-resistant subjects experienced a decrease in FF following potassium supplementation ($p<0.01$). In salt-sensitive subjects a significant decrease in glomerular filtration rate (GFR) was reported; In salt-resistant subjects, potassium supplementation had no no significant effect on GFR. Smith *et al* (1992) undertook an RCT in 21 men and women, mean age 66.5 years, given a basal diet (200 mmol sodium, 2730 mg potassium and 500 mg calcium daily) followed by supplementation with 2435 mg potassium as potassium chloride or placebo capsules daily for four days. Serum potassium and urinary potassium excretion were significantly increased by the fourth day on the potassium chloride supplemented diet. Urinary sodium excretion was also significantly higher by the fourth day on the potassium chloride supplemented diet ($p=0.02$).

Status markers

Sriboonlue *et al* (1999) undertook a ten day balance study in 15 Thai men aged 25-50 years in two areas. Subjects were given a fixed diet: the rural group had a mean potassium intake of 1731 ± 138 mg/day and the urban group had a mean intake 1839 ± 145 mg/day (not

significantly different). Faecal excretion of potassium was low in both groups, resulting in a high percentage of apparent potassium absorption in both groups. Calculated potassium balances as a result of low faecal and urinary potassium excretion were highly positive. If sweat potassium was included in the balance calculation (e.g. for the one participant in whom sweat potassium was measured), mean balance over the ten day period fell from +847 (± 373) mg/day to +396 (± 344) mg/day. If sweat potassium was taken into consideration, a higher intake of potassium was required to maintain balance. Another balance study with a mean dietary potassium intake of ~4680 mg/day, was carried out in 13 adults aged 23 to 66 years for 30 days (Tasevska *et al*, 2006). Thirty day mean urinary potassium excretions were correlated with 30 day mean analysed dietary potassium intakes ($r=0.89$, $p<0.001$). Faecal potassium was not a significant predictor of potassium intake.

Urinary markers

Urinary potassium was significantly elevated with a high potassium diet (6357 mg/day) compared to a low potassium diet (2691 mg/day) (Deriaz *et al*, 1991; **Table 7**), and there was a good correlation between potassium intake and urinary potassium output ($r=0.94$, $p<0.001$). In a two year RCT, 24 hour urinary excretion significantly increased after potassium supplementation, as potassium citrate at 823 mg potassium per day, but not at 281 mg potassium/day compared to placebo (Macdonald *et al*, 2008; **Table 5**). A study in 19 men and women aged 22-65 years randomly assigned to 1170 mg potassium as potassium chloride, 1170 mg potassium as potassium citrate or placebo for six weeks; observed mean urinary excretion significantly increased in the potassium chloride and potassium citrate intervention groups, but not in the placebo group (Braschi *et al*, 2008; **Table 3**). In an RCT in men and women aged 23-58 years consuming an initial basal diet higher in potassium (2340 mg/day potassium and 150 mmol/day sodium) followed by a basal diet low in potassium (624 mg/day potassium and 120 mmol/day sodium) with either placebo capsules or additional potassium (1785 mg potassium as potassium chloride); urinary potassium depletion was accompanied by a decrease in plasma potassium concentration from pre-study levels in the low potassium diet phase (Krishna *et al*, 1991; **Table 4**). In an RCT conducted by Gu *et al* (2001; **Table 3**), 150 adults aged 45-64 years were given 5000 mg potassium chloride daily or placebo, urinary excretion of potassium increased by 20.6 mmol/24 hour ($p<0.001$) in the supplemented group compared to placebo.

Blood markers

Barden *et al* (1991; **Table 4**) supplemented 37 women with 1622 mg potassium as potassium chloride for four days and observed a small but significant increase in serum potassium equivalent to 0.29 ± 0.03 mmol/l ($p<0.01$), Another RCT in 12 hypertensive adults aged 23 to 58 years also supplemented with 1622 mg potassium as potassium chloride, but for 12 days, and also observed a significant mean plasma potassium increase ($p<0.001$; Krishna *et al*, 1991; **Table 4**). In a study supplementing with 3164 mg/day potassium as potassium chloride or 3047 mg/day of potassium as potassium bicarbonate for eight days, both significantly increased plasma potassium compared to control periods ($p=0.01$; Sebastian *et al*, 1990). In an RCT in 15 males and females, mean age 51 years, supplemented with 1952 mg potassium as

potassium chloride or 1426 mg potassium as potassium citrate for seven days, serum potassium was significantly higher ($p<0.001$) after potassium supplementation than at baseline, but there was no significant difference between the two forms (He *et al*, 2005; **Table 4**). Braschi *et al* (2008; **Table 4**) measured changes in erythrocyte and serum potassium during a six week RCT in 19 men and women aged 22-65 years. No significant differences were seen in erythrocyte or plasma potassium concentrations after supplementation with 1170 mg potassium /day as either potassium chloride or potassium citrate. Valdes *et al* (1991; **Table 3**) undertook a four week RCT in 24 men and women with a mean age of 50 years in which serum potassium concentration significantly increased ($p<0.001$) following supplementation with 1298 mg potassium as potassium chloride. Smith *et al* (1992) undertook a four day RCT in 21 men and women (mean age 66.5 years) given a basal diet (4600 mg sodium, 2730 mg potassium and 500 mg calcium daily) followed by supplementation with 2434 mg potassium as potassium chloride or placebo capsule daily for four days. Serum potassium concentration and urinary potassium excretion were significantly increased ($p<0.001$) by the fourth day on the potassium chloride supplemented diet. Urinary sodium excretion was also significantly higher by the fourth day on the potassium chloride supplemented diet ($p=0.02$).

Ethnic differences

Six studies comparing outcome measures in black and white populations were included. Turban *et al* (2008) carried out an RCT in 413 adults consuming a fixed diet consisting of either a control diet (1.7 g potassium), a high fruit and vegetable diet (4.1 g potassium) or a diet specially formulated for a study investigating endpoints such as blood pressure, the DASH diet (4.4 g potassium). After a three week run-in period during which all participants received a low potassium control diet, a significant ethnic difference in urinary potassium concentrations was observed (mean 201 mg/day, adjusted for age, gender, and caloric intake; $p< 0.001$). At the end of intervention, the mean difference in urinary potassium excretion between white and black individuals, after adjustment for age, gender, and caloric intake, was 6 mg/day ($p = 0.95$) in the control group, 163 mg/day in the high fruit and vegetable group ($p=0.39$), and 903 mg/day in the DASH group ($p <0.001$).

In a CT 21 black and white adults (male and female) given 1217 mg potassium as potassium chloride per 70 kg bodyweight daily for three days, urinary potassium excretion was reported to be significantly lower in blacks than whites at baseline; although blacks increased urinary potassium excretion post-intervention, and they remained significantly lower than whites ($p<0.021$; Kimura *et al*, 2004). Plasma aldosterone concentrations after upright posture were significantly lower in blacks than in whites but were similar when supine, as were urinary aldosterone excretion rates. Pratt *et al* (1997) carried out an RCT supplementing with 811 mg/day potassium as potassium chloride in 24 older black and white adults (mean age ~64 years) given a low sodium diet (2000 mg/day) followed by a high sodium diet (5175 mg/day) plus 1334 mg potassium as potassium citrate) or placebo daily. After potassium supplementation, aldosterone production increased in both groups to the same extent. Potassium treatment appeared to increase lower plasma renin activity levels ($p=0.0001$). Palcios *et al* (2010) carried out a study in 50 girls aged 11-15 years and reported a lower

potassium excretion in blacks than in whites, regardless of sodium intake ($p<0.05$). There were no differences in faecal or sweat potassium excretions. A CT in 11 black and 10 white adults aged 21 to 35 years, examined electrolyte balance under strict dietary conditions (Gallen *et al*, 1998). During potassium restriction, blacks excreted less sodium than whites (862 ± 65 mmol v 1133 ± 32 mmol; $p=0.02$), while potassium excretion fell to similar levels in both groups. Cumulative excretion of potassium on the control diet was less in blacks than in whites (474 ± 79 mmol v 701 ± 34 mmol; $p=0.05$). Sodium excretion from the control diet was also significantly less in blacks than whites (1137 ± 81mmol v 1403 ± 55mmol; $p=0.02$).

Dietary interactions

Sodium

Significant increases in ecosanoid 6-keto PGF1α after four days of potassium supplementation were significantly correlated to sodium excretion, suggesting possible potassium related natriuresis which may be mediated in part by prostaglandin (Barden *et al*, 1991; **Table 4**). A CT in salt resistant normotensive volunteers aged 21-29, observed potassium (1560 mg/day) supplementation to increase diuresis and natriuresis, resulting in moderate suppression of volume expansion induced by salt loading of 410 mEq/day (Mano *et al*, 1992). In an RCT in 12 males and females aged 23-58 years consuming an initial basal diet (2340 mg/day potassium and 150 mmol/day sodium) followed by a low potassium basal diet (624 mg/day potassium and 120 mmol/day sodium) plus either placebo capsules or 3432 mg/day potassium as potassium chloride, changes in potassium intake produced alterations in urinary sodium excretion: on 3744 mg/day potassium diet; sodium excretion was 110 ± 5 mmol/day over the 10 day period. When on the 624 mg/day potassium diet, sodium excretion was 83 ± 6 mmol/day over the 10 day period ($p<0.001$; Krishna *et al*, 1991; **Table 4**). In an RCT of 11 borderline hypertensive and 10 normotensive subjects (mean age 23.5 years) on a high sodium, low potassium (9200 mg sodium, 1170 mg potassium) diet or high sodium, high potassium diet (9200 mg sodium, 3900 mg potassium) serum sodium levels were unchanged by diet (Lawton *et al*, 1990; **Table 4**). Potassium restriction (780 mg/day) significantly decreased potassium and sodium excretion immediately and they both remained low for the nine day duration of the experimental period (Gallen *et al*, 1998).

Calcium

In a CT, 10 male and female adults aged 21 to 41 years on a controlled diet containing 3315 mg/day potassium, 21.6 ± 0.9 mmol/day calcium and 165 ± 14 mmol/day sodium, were supplemented with 90 mmol/day of potassium carbonate (1966 mg potassium) or potassium chloride (1825 mg potassium) for four days. Potassium carbonate, but not potassium chloride, significantly decreased fasting and 24 hour urinary calcium excretion (Leemann *et al*, 1991). In an RCT supplementing 42 male and females adults aged 18-75 years with 1398 mg potassium as potassium bicarbonate, or 1298 mg potassium as potassium chloride, or placebo for four weeks, potassium bicarbonate decreased the 24 hour urinary calcium excretion and also the Ca:creatinine ratio significantly ($p=0.009$ and $p=0.002$, respectively; He *et al*, 2010; **Table 3**). Macdonald *et al* (2008; **Table 5**) supplemented healthy postmenopausal women

(aged 55-65 years) with potassium citrate in a 24 month RCT and observed that women on high dose (823 mg potassium/day) supplementation had significantly lower calcium excretion after three and six months supplementation (p=0.02 and 0.01, respectively), but this effect was not seen in the low dose (507 mg/day) group. In an acute one day RCT in 12 females aged 22-30 years supplemented with potassium citrate, Karp *et al* (2009; **Table 5**) observed an increase in calcium retention. The reason for the discrepancy between forms of potassium on calcium excretion is unclear and requires further research.

A CT carried out in six male and two female adults who underwent five days of potassium deprivation observed increases in both fasting and 24 hour urinary calcium excretion, whether the accompanying anion was chloride or bicarbonate (Leemann *et al*, 1991); levels returned to normal within five days of ending the period of deprivation.

Phosphorus

Sebastien *et al* (1990) observed that varying dietary potassium influenced phosphorus homeostatsis. Six healthy males aged 25-40 years on a fixed diet (140 mmol sodium, 2028 mg potassium, 9 mmol calcium, 27 mmol phosphorus per 70 kg body weight) were supplemented with 6084 mg/day as potassium bicarbonate and potassium chloride for eight days each, both caused a significant increase in serum phosphorus ($p<0.01$) and a decrease in calcitrol ($p<0.01$) compared to the control phase. Changes in serum phosphorus and plasma potassium concentrations were positively correlated ($r=0.64$; $p=0.027$).

Vitamin D

Leemann *et al* (1991) observed potassium mediated alterations in renal tubular phosphate transport and renal synthesis of $1,25(OH)_2$-vitamin D after administration with potassium salts. Serum $1,25(OH)_2$-vitamin D fell significantly during potassium bicarbonate supplementation (P-value not reported) and no effect during deprivation. Serum $1,25(OH)_2$-vitamin D increased slightly during potassium chloride deprivation (P-value unclear).

BLOOD PRESSURE (TABLE 3)

Studies including subjects with a mean systolic blood pressure (SBP) ≥ 160 mmHg or diastolic blood pressure ≥100 mmHg were not included as they were not considered to be a relevant population, as they are classified as medium-high risk for cardiovascular disease by the World Health Organisation and International Society of Hypertension (WHO, 2003). Nine studies were included, all RCTs and all assessed as being at high risk of bias. Eight of the studies were carried out in adults, one in females and seven in mixed gender populations. One of the studies was carried out in a mixed gender adolescent population.

Forty eight men and women aged 22-65 years took part in a RCT consisting of a three week run-in of controlled intakes of fruits and vegetables (providing 585 mg/day of potassium) followed by either 780 mg/day of potassium from fruit and vegetables, 1560 mg/day of

potassium from fruit and vegetables, 1560 mg/day of potassium as potassium citrate, or placebo for six weeks (Berry *et al*, 2010). There were no significant changes in ambulatory blood pressure when compared to control treatment in any of the intervention groups.

In a study in 19 men and women aged 22-65 years, randomly assigned to one of three treatments: placebo, 1170 mg potassium as potassium chloride, or 1170 mg potassium as potassium citrate daily for six weeks, there was no significant difference in blood pressure change between the two potassium supplemented groups, but both groups were significantly lower compared to placebo ($p<0.005$; Braschi *et al*, 2008).

As part of the Trials of Hypertension Prevention (TOHP), a six month RCT was undertaken in 353 male and females, aged 35-54 years, who were supplemented with 1220 mg potassium as potassium chloride or placebo daily, and it was reported that potassium supplementation was associated with a small but significant ($p=0.04$) reduction in diastolic blood pressure (DBP) after three months intervention (Culter *et al*, 1992). However, at six months, the apparent treatment effect had disappeared.

In another RCT, 150 men and women aged 45-64 years were supplemented with 1900 mg potassium as potassium chloride or placebo daily for 12 weeks (Gu *et al*, 2001). On average, the net reduction in systolic blood pressure was 5.0 mm Hg ($p<0.01$) (95% CI -2.13, -7.88 mm Hg) in the potassium intervention group compared to the placebo group; there was no significant effect on diastolic blood pressure.

In an RCT in which 42 men and women aged 18-75 years were supplemented with 1398 mg potassium as potassium bicarbonate, or 1298 mg potassium as potassium chloride, or placebo daily for four weeks, no significant differences were observed in office blood pressure among the three treatment periods, and only 24 hour and daytime systolic blood pressure (SPB) were slightly lower with potassium chloride (He *et al*, 2010). Compared with placebo, both potassium chloride and potassium bicarbonate significantly improved endothelial function as measured by brachial artery flow-mediated dilatation, increased arterial compliance as assessed by carotid-femoral pulse wave velocity, decreased left ventricular mass, and improved left ventricular diastolic function. There was no significant difference between the two potassium salts in these measurements.

In an RCT in 59 men and women, aged 25 to 65 years, supplemented with 936 mg potassium as potassium chloride or placebo daily for six weeks, the mean arterial pressure (MAP), SBP and DBP decreased significantly at six weeks in the potassium group compared to the baseline (Naismith *et al*, 2003). In the placebo group, MAP had increased significantly at six weeks compared to baseline ($p=0.005$). Compared with the placebo group, the potassium supplemented group experienced a marked and significant decrease in SBP, DBP and thus MAP.

In an RCT in 290 women (mean age 36 years), blood pressure was significantly reduced in normotensive individuals who received for 16 weeks 811 mg potassium/day as potassium chloride compared with a placebo (Sacks *et al*, 1998). Another RCT in 24 men and women with a mean age of 50 years found a significant fall in blood pressure after supplementation

with 1298 mg potassium as potassium chloride for four weeks compared with placebo (Valdes *et al*, 1991).

Forty adolescents aged 13-15 years randomly assigned to a high potassium diet (providing 3120 mg /day) or their usual diet for four weeks were studied to investigate the responses of dippers (>10% decrease in blood pressure from awake to sleep) versus non-dippers (<10% decrease in blood pressure from awake to sleep) (Wilson *et al*, 1996). According to the SBP classification 30% of the adolescents were classified as dippers. Dippers had lower sleep SBP and DBP (and mean blood pressure) than non-dippers. Dippers showed a decrease in SBP and DBP (and mean blood pressure) from baseline to post-intervention, whereas for non-dippers, blood pressure increased from baseline to post-intervention.

ALDOSTERONE AND RENIN (TABLE 4)

Studies in this section report on the effect of potassium supplementation on aldosterone and renin. Some of the studies have measured blood pressure but these data were not extracted due to study durations being less than four weeks, which is considered insufficient to demonstrate a sustained effect. In total, five studies were included, four RCTs and one CT, all of which were assessed as being at high risk of bias. The number of participants in each study was between 11 and 34. All of the studies were carried out in adults, one in females, one in males and three in mixed gender populations.

In a four day CT in 37 females (mean age ~32 years) supplemented with 1622 mg potassium as potassium chloride/day there was a small but significant increase in serum potassium equivalent to 0.29 ± 0.03 mmol/l ($p<0.01$), which was mirrored by a significant increase in plasma aldosterone (mean increase 280 ± 68.5 pmol/l, $p<0.01$) (Barden *et al*, 1991). Atrial natriuretic peptide (ANP) showed a small but significant decrease with potassium supplementation (equivalent to 1.1 pmol/l, $p<0.01$). The change in ANP was negatively correlated with change in aldosterone ($r=-0.47$, $p<0.01$, $n=29$). Coruzzi *et al* (2001) found that a low intake of potassium (702 mg/day) for 11 days caused a significant suppression of plasma aldosterone ($p<0.04$) compared to normal potassium intake (3120 mg/day) in male and female hypertensive patients aged 23-46 years. In an RCT, supplementing 15 males and females with a mean age of 51 years, with 1952 mg potassium as potassium chloride or 1426 mg potassium as potassium citrate for seven days resulted in a significantly higher plasma aldosterone with both potassium chloride and potassium citrate compared to baseline (He *et al*, 2005). In an RCT in 12 males and females, aged 23-58 years, consuming an initial basal diet (2340 mg/day potassium and 150 mmol/day sodium) followed by a low potassium basal diet (624 mg/day potassium and 120 mmol/day sodium) plus placebo capsules or 1785 mg/day potassium as potassium chloride, plasma renin activity and aldosterone decreased during the low intake of potassium compared to the high intake ($p<0.001$; Krishna *et al*, 1991). In an RCT of 11 borderline hypertensive and 10 normotensive subjects, mean age 23.5 years, on a high sodium, low potassium (9200 mg sodium, 1170 mg potassium) diet or high sodium, high potassium (9200 mg sodium, 3900 mg potassium) diet for six days, there were no significant differences in plasma aldosterone concentrations between the low and the high potassium diet

for either group (Lawton *et al*, 1990). However, both groups had decreased plasma renin activity when obtained during the standing position period on the low potassium compared to the high potassium diet ($p<0.05$). A CT in black and white adults (male and female), aged 21 to 35 years, examined electrolyte balance under strict dietary conditions for nine days; plasma renin activity and serum aldosterone levels were lower on the potassium-restricted diet in both ethnic groups ($p<0.001$; Gallen *et al*, 1998; **Table 2**). This study was assessed as being at high risk of bias.

Thus, in short term studies, potassium supplementation, irrespective of supplement form, appeared to increase plasma aldosterone concentrations, and correspondingly potassium restriction decreased aldosterone concentrations.

BONE HEALTH (TABLE 5)

Studies in this section focused on the effect of potassium on bone health. In total, six studies were included, five RCTs and one balance study, two were assessed as being at moderate risk of bias and the remaining four at high risk of bias. The number of participants in each study was between 12 and 276. Five of the studies were carried out in adults and six in adolescents. Four of the studies were carried out in females and two in mixed gender populations.

Three studies were carried out in post-menopausal women. A two year RCT in 276 post-menopausal women aged 55-65 years supplemented with low dose potassium citrate (281 mg potassium) or high dose potassium citrate (823 mg potassium), fruits and vegetables (300 g additional fruits and vegetables), or placebo daily for 24 months did not show a reduction in markers of bone turnover urinary free deoxypyridinoline cross-links (fDPD), serum N-terminal propeptide of type 1 collagen, or serum beta C-terminal telopeptide (ßCTX) or bone mineral density (BMD) loss (Macdonald *et al*, 2008). The supplement groups were double-blinded and the fruit and vegetable group was single blinded. This study was assessed as being at moderate risk of bias. Sellmeyer *et al* (2002) conducted a double-blind RCT in 52 post-menopausal women (mean age ~ 64 years) who consumed a low sodium diet (2000 mg/day) for three weeks and were then randomised to a high sodium diet (5175 mg/day) plus 1334 mg potassium as potassium citrate or a high sodium diet plus placebo daily for seven weeks. The addition of oral potassium citrate to a high salt diet prevented the increased excretion of urinary calcium and the bone resorption marker N-telopeptide caused by the high salt intake. This study was assessed as being at moderate risk of bias. A balance study in 18 post menopausal women, aged 51-77 years, gave a controlled diet (mean calcium 652 mg, phosphorus 871 mg, potassium 2300 mg, sodium 119 mmol/60 kg body weight/day) followed by supplements of potassium bicarbonate (1310-1621 mg potassium/60 kg bodyweight/day). A reduction in urinary hydroxyproline excretion in association with increased serum osteocalcin concentrations in response to the potassium bicarbonate supplements were observed, which indicates that administration of bicarbonate reduces the rate of bone resorption and increases the rate of bone formation, and may attenuate the loss of bone mass which occurs over the long-term in post-menopausal women (Sebastian *et al*, 1994). As the study used potassium bicarbonate supplements it is not possible to deduce whether the effect

was that of potassium or of the bicarbonate anion. An RCT carried out by Dawson-Hughes *et al* (2009) also included post-menopausal women, but the data for males and females were not analysed separately. This double blind RCT involved 171 men and women over 50 years of age who were supplemented with 1475 mg potassium as potassium bicarbonate , 1369 mg potassium as potassium chloride, sodium bicarbonate, or placebo daily. The biochemical bone resorption marker, N-telopeptide, was lower in the groups given bicarbonate, but not in the non-bicarbonate groups. The authors concluded that bicarbonate, but not potassium, had a favourable effect on bone resorption and calcium excretion. This study was assessed as being at high risk of bias. One study in 12 pre-menopausal women, aged 22-30 years, was an acute RCT in which 2250 mg potassium as potassium citrate or placebo was given for one day (Karp *et al*, 2009). Supplementation was reported to decrease N- terminal telopeptide of type I collagen ($p=0.045$) and increased calcium retention ($p=0.004$; Karp *et al*, 2009). However, a study of longer duration is needed to confirm this finding.

DIABETES (TABLE 6)

A prospective cohort in the USA followed 12,209 men and women for nine years, and serum potassium concentrations were measured at baseline and at three year intervals (Chatterjee *et al*, 2011). Mean serum potassium concentrations were significantly lower in blacks than in whites ($p<0.01$). There was a graded inverse relationship between serum potassium and incidence of (Type II) diabetes in both African Americans and Whites. The serum potassium-race interaction was not, however, statistically significant. When average serum potassium from baseline and three year follow up was entered into the analyses, the mean serum potassium concentration was significantly associated with risk of diabetes ($p=0.0003$) and was more strongly associated in African Americans than in whites. Thus, low normal serum potassium is associated with a greater risk of Type II diabetes and African Americans are at greater risk than whites. This study was assessed as being at high risk of bias.

RESTING METABOLIC RATE (TABLE 7)

Dériaz *et al* (1991) conducted a crossover CT in eight men, with a mean age of 26 years, to investigate the putative effects of potassium on energy expenditure. High (6357 mg/day) and low (2691 mg/day) potassium normocaloric diets were given for four days; on the fifth day after an overnight fast RMR was measured by indirect calorimetry after which subjects ingested either a placebo (high potassium diet) or 1014 mg potassium as potassium chloride (low potassium diet) and RMR measurements were repeated. Acute or chronic changes in potassium intake did not influence energy expenditure and substrate oxidation loss. However, at baseline and post supplementation, serum potassium was significantly correlated to RMR changes ($r=0.74$, $p<0.05$ for both). The study was assessed as being at high risk of bias.

SLEEP (TABLE 8)

In a double blind crossover RCT, nine healthy men aged 18-33 years consuming a low potassium diet (1590 mg/day) were supplemented with 1947 mg potassium as potassium chloride or placebo for seven days (Drennan *et al*, 1991). Potassium supplementation significantly delayed sleep-log-identified bedtime ($p<0.001$) and reduced sleep interval, as calculated from sleep log ($p<0.01$), and significantly increased sleep efficiency ($p<0.05$) due to a reduction in actigraph wake after sleep onset (WASO; $p<0.05$) compared with the placebo treatment. The results may indicate an improvement in sleep patterns with potassium supplementation, however, the study was assessed as being at high risk of bias.

BREAST MILK CONCENTRATION (TABLE 9)

All nine breast milk studies were cross-sectional. All studies were assessed as being at high risk of bias. The number of samples analysed was not always clear, but in those where it was reported, sample numbers were between 19 and 1197. Two studies reporting breast milk concentration were from countries within the European Union.

The studies reported data at various stages of lactation (0-360 days overall). Ranges were not reported, but the mean potassium concentrations reported were between 239.6 and 723 mg/l. Yamawaki *et al* (2005) determined breast milk composition from birth until one year and found that the potassium concentration in human milk was affected by stage of lactation, decreasing significantly over time; but that there was no significant change in breast milk potassium concentration between three months and one year. This was confirmed by Wack *et al* (1997) who also observed mean potassium concentration to be stable from four months postpartum until the commencement of weaning. The WHO and IAEA (Parr *et al*, 1991) investigated potassium concentrations in six different countries and found that mean concentrations varied by up to 144 mg/l between countries. Qian *et al* (2009) also identified significant differences in breast milk potassium concentrations according to geographical region ($p<0.05$), although these may have been confounded by socio-economic status. Rackicoğlu *et al* (2006) investigated the effect of Ramadan on breast milk composition and found potassium levels to be significantly higher after than during Ramadan ($p<0.001$), presumably because maternal nutrient intakes are decreased during Ramadan. The results of this study suggest that diet may influence breast milk potassium concentration.

Conclusions

Articles from January 1990 to September 2011 have been systematically searched and reviewed using a standard protocol, tailored for the specific issues relevant to potassium, with the aim of collating and assessing the body of evidence for potassium relevant to setting DRVs.

A total of 44 studies met the inclusion criteria. Health endpoints focused on metabolism and nutrient interactions, blood pressure, aldosterone and renin, bone health and breast milk potassium concentration. Potassium concentrations in breast milk are relevant for establishing requirements for infants and lactating mothers. There were limited data on diabetes, RMR and sleep. The status markers employed were serum potassium concentration and urinary potassium excretion, both of which appear to reflect short term intake.

The majority of studies were assessed as being at high risk of bias. Two moderate risk studies were included which investigated the effect of potassium on bone health. Study populations were mainly focused on adults and infants and lactating mothers. There were three studies on adolescents but no studies on children. Overall, evidence published between January 1990 and September 2011, regarding potassium in relation to the setting of DRVs appears to be of poor quality.

Table 2. Metabolism and nutrient interactions

Reference	Study type	n	Age	Sex	Intervention and control or exposure	Dietary intake estimation	Follow up time	Results: effect, mean, SD, RR/OR/HR confidence interval, etc	Overall results	Risk of bias
Gallen I *et al*, 1998	CT	21	21-35 years	10M & 11F	Experimental diet containing 20 mmol/d K (180 mmol/d Na, 158 mmol/d Cl) plus KCl supplement (80 mmol/d) or placebo plus placebo supplement	Fixed diet	9 days x 2	<u>K restricted diet</u> Serum K (mmol/l): 3.5 ± 0.1 Cumulative urinary K excretion over 9 days (mmol): 181 ± 11 Cumulative urinary Na excretion over 9 days (mmol): 984 ± 59 Body weight (kg): 71.1 ± 2.1 Total Protein (g/100ml): 6.3 ± 0.1 Hemoglobin (g/100ml): 12.5 ± 0.3 Urinary Norepinephrine (mg/d): 27.0 ± 2.0 Urinary Epinephrine (mg/d):6.0 ± 0.6 <u>K supplemented diet</u> Serum K (mmol/l): 4.1 ± 0.1 Cumulative urinary K excretion over 9 days (mmol): 576 ± 52 Cumulative urinary Na	Cumulative excretion of Na was less in blacks than whites during the K-restricted diet phase, whilst K excretion decreased to similar levels In conclusion, in healthy normotensive subjects potassium restriction was associated with increased renal sodium and chloride retention	High

Reference	Study type	n	Age	Sex	Intervention and control or exposure	Dietary intake estimation	Follow up time	Results: effect, mean, SD, RR/OR/HR confidence interval, etc	Overall results	Risk of bias
								excretion over 9 days (mmol):1256 ± 58 Body weight (kg): 69.2 ± 2.2 Total Protein (g/100ml): 6.7 ± 0.1 Hemoglobin (g/100ml): 13.2 ± 0.3 Urinary Norepinephrine (mg/d): 32.5 ± 2.4 Urinary Epinephrine (mg/d): 6.2 ± 0.7 <u>Difference (p value)</u> Serum K: 0.001 Cumulative urinary K excretion over 9 days: 0.001 Cumulative urinary Na excretion over 9 days: 0.001 Body weight: <0.001 Total Protein: 0.004 Hemoglobin: 0.002 Urinary Norepinephrine: <0.02 Urinary Epinephrine: NS		

Reference	Study type	n	Age	Sex	Intervention and control or exposure	Dietary intake estimation	Follow up time	Results: effect, mean, SD, RR/OR/HR confidence interval, etc	Overall results	Risk of bias
								Plasma renin activity and serum aldosterone levels were lower on K-restricted diet in both ethic groups ($p<0.001$). Urinary osmolarity increased from 53.0 ± 3.0 mOsm/L in the K restricted diet to 65.6 ± 3.5 mOsm/L in the K supplemented diet ($p<0.01$) in response to the water load experiment on a subset of 8 participants. Free water clearance was significantly greater during K restricted diet than K supplemented diet phase (4.44 ± 0.59 ml/min –vs- 3.72 ± 0.58 ml/min, $p=0.009$). K and Na excretion decreased immediately upon K restriction, and remained low for the duration of the experimental diet.		

Reference	Study type	n	Age	Sex	Intervention and control or exposure	Dietary intake estimation	Follow up time	Results: effect, mean, SD, RR/OR/HR confidence interval, etc	Overall results	Risk of bias
								During potassium restriction, blacks excreted less Na than whites (862 ± 65 mmol v 1133 ± 82 mmol; p=0.02), while K decreased to similar levels. Cumulative excretion of K was less in blacks than in whites on the control diet (474 ± 79 mmol v 701 ± 34 mmol; p=0.05). Na excretion was significantly less in blacks than whites on the control diet (1137 ± 81 mmol v 1403 ± 55 mmol; p=0.02)		
Kimura M *et al*, 2004	CT	73	Males: Black: 37.9 ± 7.32 years; White: 37.4 ± 3.2 years Females: Black: 36.2	31M & 42F	Supplementation of habitual diet with 60 mmol KCl/70 kg bodyweight daily. Dose provided in tablet form, to be taken after meals	Not reported	3 days	<u>Post-intervention values for KCl and control groups:</u> <u>Urinary K excretion (mmol/70 kg per day):</u> KCl: 97.0 ± 62.4 (p<0.0001-v-baseline value)	Urinary K excretion was significantly higher post-intervention in the KCl supplemented group. There	High

Reference	Study type	n	Age	Sex	Intervention and control or exposure	Dietary intake estimation	Follow up time	Results: effect, mean, SD, RR/OR/HR confidence interval, etc	Overall results	Risk of bias
			± 7.56 years; White:34.8 ± 12.3 years		with 2 glasses of water. Control subjects maintained habitual diet, and drank 2 glasses of water with each meal (no placebo tablet)			Control: 46.0 ± 19.0 In whites: KCl: 119.71.5 ± 71.5 (p=0.0002-v-baseline value) control: 51.3 ± 20.1 In blacks: KCl: 72.0 ± 38.9 (p=0.021-v-baseline value) control: 38.5 ± 14.9 Platelet reactivity (ADP-mediated platelet aggregation, EC_{50}, mmol/l): KCl: 1.18 ± 0.383 (p<0.0017-v-baseline value) Control: 1.02 ± 0.289	was no significant increase in urinary K in the control group. Urinary K excretion was significantly lower in blacks than whites at baseline; although blacks increased urinary K excretion post-intervention, they were still significantly lower than whites (p<0.021) No differences in serum K between control and KCl groups after supplementation. K supplemented	

Reference	Study type	n	Age	Sex	Intervention and control or exposure	Dietary intake estimation	Follow up time	Results: effect, mean, SD, RR/OR/HR confidence interval, etc	Overall results	Risk of bias
									blacks exhibited increase in EC_{50} between baseline and end (1.18 ± 0.393 µmol/l at baseline to 1.27 ± 0.442 µmol/l at end). However, difference in EC_{50} change between K-supplemented and control blacks was not statistically significant (p=0.17) Thus, K supplementation for 3 days diminished platelet activity (as seen by increase in EC_{50} of the ADP-evoked platelet aggregation)	

Reference	Study type	n	Age	Sex	Intervention and control or exposure	Dietary intake estimation	Follow up time	Results: effect, mean, SD, RR/OR/HR confidence interval, etc	Overall results	Risk of bias
Lemann Jr J *et al*, 1991	CT	10/8	21-41 years	8M & 2F	<u>K supplementation study</u> Constant wholefood diet (K 85 ± 6, Ca 21.6 ± 0.9, Na 165 ±14 mmol/d); After initial control period(4 days), 90 mmol/d of either KCl, or $KHCO_3$ <u>K deprivation study</u> Liquid diet formula containing either $KHCO_3$ at 1 mmol/kg/d (n=4) or KCl at 1 mmol/kg/d (n=4). On this controlled diet for 5 days, followed by 5 day deprivation period where the KCl or $KHCO_3$	Fixed diet	8-12 days 10-11 days	<u>K supplementation</u> On day 4 of supplementation daily urinary Ca excretion and fasting U_{Ca} V/GFR were lower for KHCO3 than during control (-1.26 ± 0.29; p<0.005 and 0.0069 ± 0.0019 mmol/l GFR; p=0.005). Administration of $KHCO_3$ was accompanied by significant increases in total serum Ca after breakfast & fasting PO_4 concentrations <u>K deprivation</u> Both dietary KCl and $KHCO_3$ deprivation (mean reduction in dietary K intake -67 ± 8 mmol/d) were accompanied by an increase in daily urinary Ca excretion and fasting U_{Ca} V/GFR that averaged on the 5^{th} day +1.31 ± 0.25	$KHCO_3$ decreases fasting & 24 hr urinary Ca excretion. K deprivation increases both fasting and 24 hr urinary Ca excretion whether the accompanying anion is Cl^- or HCO_3^- K mediated alterations in renal tubular phosphate transport and renal synthesis of $1,25(OH)_2$-vitamin D were observed	High

Reference	Study type	n	Age	Sex	Intervention and control or exposure	Dietary intake estimation	Follow up time	Results: effect, mean, SD, RR/OR/HR confidence interval, etc	Overall results	Risk of bias
					removed from diet formula. Followed by recovery period of 5 days ($KHCO_3$) or 6 days (KCl) where the K salts added back to diet			mmol/d ($p<0.005$) and +0.0069 ± 0.0012 mmol/l GFR ($p<0.005$) above control. Both daily urinary Ca excretion & fasting U_{Ca} V/GFR returned toward or to control concentrations at the end of recovery. Serum $1,25(OH)_2$-vit D levels fell slightly but significantly during $KHCO_3$ administration, and rose slightly during dietary KCl deprivation (but no changed detected during $KHCO_3$ deprivation)		
Mano M *et al*, 1992	CT	12	21-29 years	M	Initial low salt period (LSB): 40 mEq/d K and 60 mEq/d Na (5 days) in both groups, followed by increased Na by 350 mEq/d in both groups (high salt period, HSP)	Fixed diet	10 days	**<u>Basal levels (LSP)</u>** <u>Control group</u> Hct (%): 45.3 ± 0.6 MCV (μm^3): 91.2v±1.3 serum Na (mEq/l):139.8 ± 0.4 serum K (mEq/l): 4.0 ± 0.1 serum NE (nmol/l):	Serum K concentration significantly increased from basal level in the K supplemented group on the 1st, 2nd, and 3rd days of HSP after which it	High

Reference	Study type	n	Age	Sex	Intervention and control or exposure	Dietary intake estimation	Follow up time	Results: effect, mean, SD, RR/OR/HR confidence interval, etc	Overall results	Risk of bias
					and 100 mEq/d K (as KCl) in one group only			1.43 ± 0.18 <u>K group</u> Hct (%): 47.5 ± 1.1 MCV (μm^3):89.8 ± 2.2 serum Na (mEq/l): 139.1 ± 0.2 serum K (mEq/l): 4.0 ± 0.1 serum NE (nmol/l):1.73 ± 0.21 <u>HSP (day 5)</u> <u>Control group</u> Hct (%): 39.7 ± 0.9 MCV (µm3): 90.9 ± 1.3 serum Na (mEq/l): 140.7 ± 0.8 serum K (mEq/l): 37 ± 0.1 serum NE (nmol/l): 0.42 ± 0.04 <u>K group</u> Hct (%): 43.8 ± 1.1 MCV (μm^3): 90.0 ± 2.1 serum Na (mEq/l):139.5 ± 0.3	gradually decreased to basal level. In salt resistant normotensives K supplementation increased diuresis and natriuresis, resulting in moderate suppression of volume expansion induced by salt loading.	

Reference	Study type	n	Age	Sex	Intervention and control or exposure	Dietary intake estimation	Follow up time	Results: effect, mean, SD, RR/OR/HR confidence interval, etc	Overall results	Risk of bias
								serum K (mEq/l): 4.2 ± 0.1 Serum NE (nmol/l): 0.73 ± 0.10 Serum Na significantly increased in the control group during HSP ($p<0.05$), however there was NS serum Na increase in the K supplemented group Serum K concentration significantly increased from basal level in the K supplemented group on the 1st, 2nd, and 3rd days of HSP ($p<0.05$) after which it gradually decreased to basal level Plasma NE gradually decreased during HSP in both groups ($p<0.001$), on the 3rd day the decrease from basal level was not significant in the K supplemented group		

Reference	Study type	n	Age	Sex	Intervention and control or exposure	Dietary intake estimation	Follow up time	Results: effect, mean, SD, RR/OR/HR confidence interval, etc	Overall results	Risk of bias
								but was significant in the control group ($p<0.005$). On the 5th day decreases from basal were significant in both groups ($p<0.005$)		
Palcios C *et al*, 2010	Balance study	50	11-15 years	F	1950 mg dietary K plus low Na diet (57 mmol/l Na) or high Na diet (174 mmol/l Na) daily	Duplicate diet & 6 day dietary records	20 days	<u>Baseline</u> K intake (mmol/l/d, mean ± SD) Blacks: 59.6 ± 6.2 Whites: 64.9 ± 5.9 Na intake (mmol/l/d, mean ± SD) Blacks: 121.5 ± 8.5 Whites: 122.0 ± 7.5 Urinary K excretion (mmol/l/d, mean ± SD) Blacks: 36.5 ± 6.0 Whites: 37.0 ± 5.3 <u>High Na diet</u> Urinary Na excretion (mmol/l/d) Black: 130.3 ± 21.0 White: 158.8 ± 15.4 ($p<0.05$ compared to blacks) Sweat K (mmol/l/d)	Urinary K excretion was lower in blacks than in whites, regardless of Na intake ($P< 0.05$), with no differences in faecal or sweat K excretion. Cumulative K retention was significantly higher in blacks while consuming the low Na diet. Plasma aldosterone concentrations after upright posture were significantly	High

Reference	Study type	n	Age	Sex	Intervention and control or exposure	Dietary intake estimation	Follow up time	Results: effect, mean, SD, RR/OR/HR confidence interval, etc	Overall results	Risk of bias
								Black: 5.55 ± 2.39 White: 5.69 ± 2.46 ($p<0.05$ compared to Low Na diet group) Sweat Na (mmol/l/d) Black: 5.43 ± 2.06 White: 7.50 ± 7.31 Serum Na (mEq/l) Black: 140.8 ± 3.07 White: 143.3 ± 2.86 Serum K (mEq/l) Black: 4.44 ± 0.38 White: 4.32 ± 0.37 <u>Low Na Diet</u> Urinary Na excretion (mmol/l/d) Black: 66.7 ± 10.6 White: 73.6 ± 12.8 Sweat K (mmol/l/d) Black: 5.41 ± 1.96 White: 5.42 ± 1.83 Sweat Na (mmol/l/d) Black: 3.96 ± 1.87 White: 4.28 ± 2.30 Serum Na (mEq/l) Black: 140.9 ± 2.37 White: 142.2 ± 1.50 Serum K (mEq/l) Black: 4.46 ± 0.25	lower in blacks than in whites but were similar when supine, as were urinary aldosterone excretion rates. On week 3, BP, body weight, urinary volume, creatinine, and serum Na and K were similar	

Reference	Study type	n	Age	Sex	Intervention and control or exposure	Dietary intake estimation	Follow up time	Results: effect, mean, SD, RR/OR/HR confidence interval, etc	Overall results	Risk of bias
								White: 4.38 ± 0.31		
Pratt J *et al*, 1997	RCT	24	Placebo: 63 ± 8 years K citrate: 65 ± 8 years	10M & 14F	Low sodium diet (2000mg/d) followed by High sodium diet (5175 mg/d) plus 1334 mg K (as K citrate) or placebo daily	Not reported	9 days	<u>KCl group</u> <u>Blacks</u> Serum Na (mmol/l): 137.4 ± 1.2 Serum K (mmol/l): 4.3 ± 0.2 Na excretion (mmol/24 hr): 160.1 ± 43.2 K excretion (mmol/24 hr): 71.8 ± 9.4 Plasma renin activity (ng/l per s) 0700hr: 0.824 ± 1.007 0900hr: 1.471 ± 0.818 Plasma aldosterone (pmol/l) 0700hr: 310.4 ± 206.5 0900hr: 869.4 ± 323.1 Aldosterone excretion (nmol/24 hr): 12.30 ± 8.47 <u>Whites</u> Serum Na (mmol/l): 139.0 ± 2.3 Serum K (mmol/l): 4.3 ± 0.3 Na excretion (mmol/24	After supplementing the intake of potassium, aldosterone production increased in black and white subjects to the same extent. K treatment appeared to increase lower plasma renin activity levels (p=0.0001)	High

Reference	Study type	n	Age	Sex	Intervention and control or exposure	Dietary intake estimation	Follow up time	Results: effect, mean, SD, RR/OR/HR confidence interval, etc	Overall results	Risk of bias
								hr): 176.5 ± 48.6 K excretion (mmol/24 hr): 74.3 ± 15.0 Plasma renin activity (ng/l per s) 0700hr: 0.774 ± 0.605 0900hr: 2.002 ± 1.546 Plasma aldosterone (pmol/l) 0700hr: 280.4 ± 159.0 0900hr: 938.8 ± 437.5 Aldosterone excretion (nmol/24 hr): 10.32 ± 7.43 <u>Placebo group</u> <u>Blacks</u> Serum Na (mmol/l): 135.3 ± 5.1 Serum K (mmol/l): 44 ± 0.2 Na excretion (mmol/24 hr): 165.8 ± 44.2 K excretion (mmol/24 hr): 37.1 ± 12.2 Plasma renin activity (ng/l per s) 0700hr: 0.379 ± 0.154 0900hr: 1.229 ± 0.500 Plasma aldosterone		

Reference	Study type	n	Age	Sex	Intervention and control or exposure	Dietary intake estimation	Follow up time	Results: effect, mean, SD, RR/OR/HR confidence interval, etc	Overall results	Risk of bias
								(pmol/l) 0700hr: 157.2 ± 129.8 0900hr: 420.9 ± 288.8 Aldosterone excretion (nmol/24 hr): 5.00 ± 4.90 <u>Whites</u> Serum Na (mmol/l): 139.5 ± 1.6 Serum K (mmol/l): 4.3 ± 0.3 Na excretion (mmol/24 hr): 152.3 ± 46.8 K excretion (mmol/24 hr): 44.0 ± 9.8 Plasma renin activity (ng/l per s) 0700hr: 0.793±0.546 0900hr: 2.424±1.315 Plasma aldosterone (pmol/l) 0700hr: 246.0 ± 208.3 0900hr: 705.6 ± 397.7 Aldosterone excretion (nmol/24 hr): 5.75 ± 2.86		
Schmidlin O *et al*,	CT	16	25-60 years	14M & 2F	Initial basal diet low NaCl, low K (15 mmol Na &	Fixed diet	21 days	*HH (high NaCl/high K) dietary group-vs-HL (high NaCl/low K)*	K bicarbonate increased serum K in both Salt-	High

Reference	Study type	n	Age	Sex	Intervention and control or exposure	Dietary intake estimation	Follow up time	Results: effect, mean, SD, RR/OR/HR confidence interval, etc	Overall results	Risk of bias
1999					30 mmol K per 70 kg body weight); followed by High NaCl, low K (250 mmol/70 kg/d NaCl & 30 mmol K/70 kg/d) and high NaCl, high K (high NaCl diet plus 140 mmol/70 kg/d $KHCO_3$)			*dietary group in salt sensitive (SS) and salt resistant (SRE) subjects:* Serum K, SS subjects, mmol/l: HH: 4.39 ± 0.15; HL: 3.36 ± 0.05 ($p<0.01$ HL-vHH) Serum K, SRE subjects, mmol/l: HH:4.37 ± 0.19; HL: SR: 3.36 ± 0.06 ($p<0.01$ HL-v-HH) Urinary K excretion, SS subjects, mmol/d: HH: 155 ± 8; HL: 29 ± 2 ($p<0.01$ HL-v-HH) Urinary K excretion, SRE subjects, mmol/d: HH: 132 ± 14; HL: 26 ±2 ($p<0.01$ HL-v-HH) GFR, SS subjects, ml/min: HH: 89 ± 4; HL: 108 ±5 ($p<0.01$ HL-v-HH) GFR SRE subjects, ml/min: HH: 100 ± 8; HL: 111 ± 7	resistant and salt-sensitive individuals. Urinary K excretion increased following high K diet in both SS and SRE individuals. Dietary K supplementation reversed the NaCl induced increases in renal vascular resistance and filtration fraction (FF) observed in SS subjects. In SRE subjects, K supplementation induced a small but not significantly different decrease in GFR; in SS subjects a	

Reference	Study type	n	Age	Sex	Intervention and control or exposure	Dietary intake estimation	Follow up time	Results: effect, mean, SD, RR/OR/HR confidence interval, etc	Overall results	Risk of bias
								FF, SS subjects, %: HH: 18.6 ± 1.1; HL: 21.4 ± 1.1 ($p<0.01$ HL-v-HH) FF, SRE subjects, %: HH: 24.2 ± 2.0; HL: 23.8 ± 2.1	significant decrease in GFR was reported. SS and SRE subjects experienced similar effects on FF following K supplementation (SS: -2.8 ± 0.5%; SRE: 0.4 ± 1, $p<0.01$)	
Sebastian A *et al*, 1990	CT	6	25-40 years	M	52mmol K/70 kg body weight for initial equilibrium period (5-10 days), followed by control period (7 days), followed by 156 mmol K/70 kg body weight (as $KHCO_3$), then recovery period (no $KHCO_3$), followed by 156 mmol/70 kg body	Fixed diet	31 days	Control period Plasma K (mmol/l): 3.98 ± 0.03 Plasma Cl (mEq/l): 106 ± 0.5 Serum PTH (pg/ml): 12 ± 0.6 Serum Pi (mmol/l): 1.17 ± 0.02 Serum Ca (mmol/l): 1.15 ± 0.02 Calcitrol (pg/ml): 36±4 KHCO3 period Plasma K (mmol/l): 4.07 ± 0.03	K supplementation increased serum Pi and decreased calcitrol. Serum K significantly increased with K supplementation.	High

Reference	Study type	n	Age	Sex	Intervention and control or exposure	Dietary intake estimation	Follow up time	Results: effect, mean, SD, RR/OR/HR confidence interval, etc	Overall results	Risk of bias
					weight (as KCl)			Plasma Cl (mEq/l): 105 ± 0.5 Serum PTH (pg/ml): 13 ± 0.6 Serum Pi (mmol/l): 1.25 ± 0.03 Serum Ca (mmol/l): 1.16 ± 0.01 Calcitrol (pg/ml): 30 ± 3 <u>Recovery period</u> Plasma K (mmol/l):3.90 ± 0.03 Plasma Cl (mEq/l): 107 ± 0.7 Serum PTH (pg/ml):12 ± 0.7 Serum Pi (mmol/l): 1.17 ± 0.04 Serum Ca (mmol/l):1.15 ± 0.01 Calcitrol (pg/ml): 39 ± 3 <u>KCl period</u> Plasma K (mmol/l): 4.06 ± 0.04 Plasma Cl (mEq/l): 106 ± 7		

Reference	Study type	n	Age	Sex	Intervention and control or exposure	Dietary intake estimation	Follow up time	Results: effect, mean, SD, RR/OR/HR confidence interval, etc	Overall results	Risk of bias
								Serum PTH (pg/ml): 12 ± 0.08 Serum Pi (mmol/l): 1.25 ± 0.03 Serum Ca (mmol/l): 1.16 ± 0.01 Calcitrol (pg/ml): 32 ± 4 K supplementation increased serum Pi ($p<0.01$) and decreased calcitrol ($p=0.02$). Serum K significantly increased with K supplementation. There were no significant differences among periods for serum PTH, plasma renin activity, body weight, serum albumin or creatinine clearance. Changes in serum phosphorus and plasma potassium concentrations were		

Reference	Study type	n	Age	Sex	Intervention and control or exposure	Dietary intake estimation	Follow up time	Results: effect, mean, SD, RR/OR/HR confidence interval, etc	Overall results	Risk of bias
								correlated positively when dietary potassium intake was changed within the normal range (r=0.64; p=0.027).		
Smith S *et al*, 1992	RCT	21	66.5 ± 5.8 years (mean ± SD)	12M & 9F	Basal diet (200 mmol Na, 70 mmol K & 500 mg Ca daily) followed by supplementation KCl, (120 mmol) or placebo capsule daily	Dietary history	4 days	<u>Serum K (mmol/l) comparisons on KCl or placebo diet:</u> KCl: 4.3 ± 0.1; Placebo: 3.9 ± 0.1 p=0.002 <u>Urinary K excretion (mmol/d) comparisons on KCl or placebo diet:</u> KCl: 179.4 ± 4; Placebo: 70 ± 4 p=0.0001	Serum K and urinary K excretion were significantly increased by the 4th day on the KCl supplemented diet Urinary Na excretion was also significantly higher by the 4th day on the KCl supplemented diet (p=0.02)	High
Sriboonlue P *et al*, 1999	Balance study	15	25-50 years	M	Rural (R) group mean intake 1731 ± 138 mg/d Urban (U) group mean intake 1839	Fixed diet	10 days	Day 1 and day 10 serum K for R and U groups, mEq/L (mg/l) R group: Day1: 3.8 ± 0.3 (148.2	Faecal and urinary excretion of K was low in both groups, resulting in high	High

Reference	Study type	n	Age	Sex	Intervention and control or exposure	Dietary intake estimation	Follow up time	Results: effect, mean, SD, RR/OR/HR confidence interval, etc	Overall results	Risk of bias
					± 145 mg/d			± 11.7) Day 10: 3.7 ± 0.3 (144.3 ± 11.7) U group: Day1: 4.1 ± 0.3 (159.9 ± 11.7) Day 10: 3.9 ± 0.3 (152.1 ± 11.7) Pooled K balance data across the 10 day study period (mean ± SD): R group: K intake (mg/d): 1731 ± 138 Urinary K (mg/d): 721 ± 29 Faecal K (mg/d): 148 ± 25 K Balance (not mean/SD): +860 ± 140 % K absorption (%): 91.31 ± 1.48 U group: K intake (mg/d): 1839 ± 145 Urinary K (mg/d): 919 ± 186 Faecal K (mg/d): 164 ± 21	K absorption for both groups. Calculated K balances as a result of low faecal and urinary K excretion were highly positive. If sweat K is included in balance calculation (e.g. for the one participant in whom sweat K was measured), mean balance over the 10 day period reduced from +847 ± 373 to +396 ± 344 mg/d Mean K intakes of rural and Urban groups were not significantly different, and were both lower	

Reference	Study type	n	Age	Sex	Intervention and control or exposure	Dietary intake estimation	Follow up time	Results: effect, mean, SD, RR/OR/HR confidence interval, etc	Overall results	Risk of bias
								K balance: +756 ± 222 % apparent absorption (%): 90.97 ± 1.67 K balance data for one participant in whom sweat K excretion was measured (mean ±SD over 10 day study period): Sweat K (mg/d): 451 ± 57 Balance (not considering sweat): + 847 ± 373 Balance (considering sweat K excretion): +396 ± 344	than the range of the estimated safe and adequate daily dietary intake (ESADI) of USA (1875-5625 mg/d). There were no significant changes in serum K across study period, suggesting an uptake of K as if participants had subclinical intracellular K deficiency. If sweat K is taken into consideration, a higher intake of K is required to maintain balance	
Tasevska N *et al*, 2006	Balance study	13	23-66 years	7M & 6F	Dietary K (mean ~122 mmol/d)	Dietary records & Duplicate	30 days	<u>Mean Dietary K intake</u> Analysed: 121.3 mmol/d Calculated: 122.7	30 day mean urinary K excretions were correlated with	High

Reference	Study type	n	Age	Sex	Intervention and control or exposure	Dietary intake estimation	Follow up time	Results: effect, mean, SD, RR/OR/HR confidence interval, etc	Overall results	Risk of bias
						diets		mmol/d correlation between analysed and calculated K intakes: 0.98 ($p<0.001$) Mean urinary K: 92.7 mmol/d % K recovery: 77.0 ± 6.7 % (95% CI= 72.8-81.0 %) Mean faecal K =10.9 mmol/100 g Fecal K recovery: 17.5 ±4 .6 % (95% CI 14.8-20.3%) <u>Percentage of dietary K recovery (when stool and urine mean measurements are summed):</u> 94.4 ± 7.1 % (95% CI 90.1-98.7 %)	30 day mean analysed dietary K intakes ($r=0.89$ $p<0.001$) 30 day mean urinary K excretions were correlated with 30 day mean estimated dietary K intakes ($r=0.90$ $p<0.001$) Urinary K was the main predictor of K intake (adjusted $R^2=0.85$, $p<0.001$) Faecal K was not a significant predictor of K intake In these subjects, who were consuming their	

Reference	Study type	n	Age	Sex	Intervention and control or exposure	Dietary intake estimation	Follow up time	Results: effect, mean, SD, RR/OR/HR confidence interval, etc	Overall results	Risk of bias
									habitual diet in a strictly controlled environment, where all intakes were measured for 30 d, the mean K intake was 121.3 mmol/d and was comparable to K intake of other studies of similar design. Urinary K was comparable to urinary Nitrogen as a biomarker of intake	
Turban S *et al*, 2008	RCT	413	≥22 years	208M & 205F	Control diet (1.7 g/d K), Fruit/ Vegetable (F/V) diet, 4.1 g/d K or DASH diet, 4.4 g/d K	Fixed diet	8 weeks	Percentage of dietary K and Na excreted in urine (urinary excretion (mg/d)/dietary intake (mg/d))*100 <u>K (%; mean ± SD)</u> <u>White</u> End of control period:	After a 3-week run-in period during which all participants received a low-K control diet, a significant ethnic difference remained (mean 201 mg/d,	High

Reference	Study type	n	Age	Sex	Intervention and control or exposure	Dietary intake estimation	Follow up time	Results: effect, mean, SD, RR/OR/HR confidence interval, etc	Overall results	Risk of bias
								74 ± 25 End of intervention, control diet: 73 ± 27 End of intervention, F/V diet: 60 ± 21 End of intervention, DASH diet: 69 ± 17 <u>Black</u> End of control period: 67 ± 24 End of intervention, control diet: 74 ± 24 End of intervention, F/V diet: 53 ± 19 End of intervention, DASH diet: 50 ± 18 <u>Na (%; mean ± SD)</u> <u>White</u> End of control period: 91 ± 28 End of intervention, control diet: 88 ± 34 End of intervention, F/V diet: 83 ± 29 End of intervention, DASH diet: 90 ± 28 <u>Black</u> End of control period: 92 ± 32	adjusted for age, gender, and caloric intake; P < 0.001). At the end of intervention, the mean difference in urinary K in white compared with black individuals after adjustment for age, gender, and caloric intake was 6 mg/d (P = 0.95) in the control group, 163 mg/d in the fruits/vegetables group (P = 0.39), and 903 mg/d in the DASH group (P <0.001)	

Reference	Study type	n	Age	Sex	Intervention and control or exposure	Dietary intake estimation	Follow up time	Results: effect, mean, SD, RR/OR/HR confidence interval, etc	Overall results	Risk of bias
								End of intervention, control diet: 91 ± 31 End of intervention, F/V diet: 88 ± 38 End of intervention, DASH diet: 92 ± 46		

Table 3. Blood pressure

Reference	Study type	n	Age	Sex	Intervention and control or exposure	Dietary intake estimation	Follow up time	Results: effect, mean, SD, RR/OR/HR confidence interval, etc	Overall results	Risk of bias
Berry S *et al*, 2010	RCT	48	22-65 years	23M & 25F	3 week run-in of controlled level of fruit and vegetable intake (providing 15 mmol/d K) followed by either: 1) 20 mmol/d K as fruit & vegetables, 2) 40 mmol/d K as fruit & vegetables, 3) 40 mmol/d K (as K citrate), or 4) placebo	Food record cards (fruit & vegetables only)	6 weeks	<u>Urinary K (mmol/d; mean, 95% CI):</u> 20 mmol/d Fruit & vegetables: 75 (67, 83) p<0.05-v-placebo 40 mmol/d fruit & vegetables: 84 (74, 94) p<0.05-v-placebo K citrate (40 mmol/d): 87 (77,96) p<0.05-v-placebo Placebo: 60 (52, 67) Urinary Na:K significantly higher in 40 mmol K/d as fruit & vegetables and 40 mmol as K citrate (p<0.05) compared to placebo, NS between 20 mmol K/d as fruit & vegetable compared to placebo <u>Mean change from placebo group in intervention groups</u>: <u>Ambulatory SBP:</u> 20 mmol/d fruit & vegetables: 0.8 (-3.5,	The increase in urinary K excretion from 40 mmol/d K provided by fruit and vegetables did not differ from that obtained from 40 mmol/d K provided by K-citrate supplement Changes in ambulatory BP when compared to control treatment were not statistically different in any of the intervention groups There was no evidence to indicate that higher intakes of K provided by either supplementation	High

Reference	Study type	n	Age	Sex	Intervention and control or exposure	Dietary intake estimation	Follow up time	Results: effect, mean, SD, RR/OR/HR confidence interval, etc	Overall results	Risk of bias
								5.0) 40 mmol/d fruit & vegetables: 1.7 (-3.0, 5.3) 40 mmol K citrate: 1.8 (-2.1, 5.8) <u>Ambulatory DBP:</u> 20 mmol/d fruit & vegetables: 0.8 (-1.9, 3.5) 40 mmol/d fruit & vegetables: 1.5 (-1.5, 4.4) 40 mmol/d K citrate: 1.4 (-1.6, 4.4) <u>Pulse wave velocity (PWV; m/s):</u> 20 mmol/d fruit & vegetables: 0.1 (-0.3, 0.4) 40 mmol/d fruit & vegetables: 0.0 (-0.3, 0.3) 40 mmol/d K citrate: 0 (-2.8, 2.8) <u>Flow mediated dilation (FMD; %):</u> 20 mmol/d fruit & vegetables: 0.1 (-0.6, 0.8) 40 mmol/d fruit &	or increased intakes of fruit and vegetables resulted in lower BP or improvements to vascular function	

Reference	Study type	n	Age	Sex	Intervention and control or exposure	Dietary intake estimation	Follow up time	Results: effect, mean, SD, RR/OR/HR confidence interval, etc	Overall results	Risk of bias
								vegetables: 0.3 (-0.5, 1.0) 40 mmol/d K citrate: -0.1 (-0.9, 0.7) In female participants, mean serum intracellular adhesion molecule-1 was significantly greater ($p<0.05$) in the treatment providing 40 mmol/d K as fruit and vegetables than in the control treatment, but NS between the male participants		
Braschi A *et al*, 2008	RCT	90	22-65 years	32M & 58F	3 groups, randomly assigned (n at baseline): 1) placebo (n=42) 2) K chloride (30 mmol/d K) (n=34) 3) K citrate (30 mmol/d K) (n=33)	Not reported	6 weeks	*Changes in BP (comparison of changes between K groups and placebo group):* <u>K chloride-vs-placebo (final from baseline; mean ±SEM):</u> SBP: -5.24 ± 1.10, 95% CI -7.43, -3.06 ($p<0.005$ from zero) DBP: -4.30 ± 1.05, 95% CI -6.39, -2.20 ($p<0.005$ from zero) MAP: -4.70 ± 0.94,	Baseline mean urinary K did not differ significantly between groups. At end of supplementation period, mean urinary excretion significantly increased in the K chloride & K citrate intervention groups, but not in	High

Reference	Study type	n	Age	Sex	Intervention and control or exposure	Dietary intake estimation	Follow up time	Results: effect, mean, SD, RR/OR/HR confidence interval, etc	Overall results	Risk of bias
								95% CI -6.56, -2.84 (p<0.005 from zero) <u>K-citrate-vs-placebo (final from baseline; mean ± SEM):</u> SBP: -6.69 ± 1.09, 95% CI -8.85, -4.53 (p<0.005 from zero) DBP: -4.26 ± 1.03, 95% CI -6.31, -2.21 (p<0.005 from zero) MAP: -5.22 ± 0.92, 95% CI -7.04, -3.39 (p<0.005 from zero) <u>Increase in Urinary K from baseline:</u> K-citrate group: 24.98 ± 8.47 mmol/d (95% CI 8.12, 41.85, p<0.01) K chloride group: 22.60 ± 8.91 mmol/d (95% CI 4.86, 40.34, p<0.01) (p values are comparisons with placebo group)	the placebo group. Baseline plasma & erythrocyte K were similar in all three groups at baseline. At the end of 6 weeks, there were NS changes in plasma or erythrocyte K No difference in BP change between the two K supplementation groups, but both supplementation groups were significantly different to placebo	
Culter J *et al*, 1992 Whelton P	RCT	353	35-54 years	225M & 98F	1220 mg/d K (as K chloride; n=178) or	FFQ & 24 hr recall	6 months	<u>Placebo (mean ± SD)</u> Change in urinary K excretion (mmol/24 hr)	Compared to placebo, active treatment was	High

Reference	Study type	n	Age	Sex	Intervention and control or exposure	Dietary intake estimation	Follow up time	Results: effect, mean, SD, RR/OR/HR confidence interval, etc	Overall results	Risk of bias
et al, 1995; Whelton P *et al*, 1997					placebo (n=175)			3 month: -2.4 ± 27.6 6 month: -4.9 ± 27.9 Average (3, 6 month): -3.1 ± 25.0 Change in urinary Na excretion (mmol/24 hr) 3 month: -8.1 ± 79.7 6 month: -12.7 ± 80.1 Average (3, 6 month): -6.9 ± 75.6 Diastolic BP (mmHg) 3 month: p<0.005 6 month: NS Average (3, 6 month): NS Change in systolic BP (mmHg) 3 month: NS 6 month: NS Average (3, 6 month): NS <u>Potassium (mean ± SD)</u> Change in urinary K excretion (mmol/24 hr) 3 month: 41.6 ± 39.7 6 month: 37.4 ±38.5 Average (3, 6 month): 38.9 ± 33.3 Change in urinary Na excretion (mmol/24 hr) 3 month: -8.2 ± 75.6	associated with a small but significant (p = 0.04) reduction in diastolic BP following 3 months of therapy. Following 6 months, however, this apparent treatment effect had virtually disappeared (mean reduction in diastolic blood pressure = 0.3 mmHg). NS effect of K supplementation on systolic BP at either follow-up visit. There was a significant, independent, dose-response relationship between change in both 24 hr urinary potassium excretion &	

Reference	Study type	n	Age	Sex	Intervention and control or exposure	Dietary intake estimation	Follow up time	Results: effect, mean, SD, RR/OR/HR confidence interval, etc	Overall results	Risk of bias
								6 month: -6.3 ± 74.3 Average (3, 6 month): -6.6 ± 64.4 Diastolic BP (mmHg) 3 month: 0.23 ± 5.50 (n=168) 6 month: -0.03 ± 4.50 Average (3, 6 month): 0.28 ± 4.50 Change in systolic BP (mmHg) 3 month: -0.14 ± 7.27 6 month: -1.00 ± 5.69 Average (3, 6 month): -0.50 ± 5.48	urinary Na:K ratio and the corresponding change in diastolic BP	
Gu D *et al*, 2001	RCT	150	45-64 years	60M & 90F	K chloride (0.5 g/d) or placebo	Not reported	12 weeks	<u>Change data for K supplement (mean ± SD):</u> *Urinary K excretion, mmol/24hr:* 6 weeks: 21.2 ± 28.8 12 weeks: 18.4 ± 28.9 Average change: 19.4 ± 25.0 Change in systolic and diastolic BP from baseline: *Change in systolic BP (mmHg):* 6 weeks: -12.1 ± 11.4	Compared to placebo, K supplementation was associated with significant reduction in systolic BP, but not diastolic	High

Reference	Study type	n	Age	Sex	Intervention and control or exposure	Dietary intake estimation	Follow up time	Results: effect, mean, SD, RR/OR/HR confidence interval, etc	Overall results	Risk of bias
								12 weeks: -13.1 ± 10.7 average change: -12.5 ± 9.8 *Change in diastolic BP (mmHg):* 6 weeks: -1.4 ± 7.4 12 weeks: -3.8 ± 6.2 Average change: -2 <u>Change data for placebo (mean ± SD):</u> *Urinary K excretion, mmol/24hr:* 6 weeks: 0.2 ± 15.2 12 weeks: -2.2 ± 13.6 average change: -1.2 ± 10.9 Change in systolic and diastolic BP from baseline: *Change in systolic BP (mmHg):* 6 weeks: -5.7 ± 10.6 12 weeks: -9.4 ± 9.0 average change: -7.5 ± 7.5 *Change in diastolic BP (mmHg):* 6 weeks: -0.2 ± 6.5 12 weeks: -3.7 ± 6.0 average change: -1.9 ±		

Reference	Study type	n	Age	Sex	Intervention and control or exposure	Dietary intake estimation	Follow up time	Results: effect, mean, SD, RR/OR/HR confidence interval, etc	Overall results	Risk of bias
								5.1 On average, net reduction in systolic BP was 5.0 mmHg ($p<0.01$) (95%CI -2.13–7.88 mmHg) in the K intervention group compared to placebo group Average net increase in urinary excretion of K was 20.6 mmol/24 hr during the 12 week intervention compared to placebo control group ($p<0.001$) In active compared to placebo group, urinary excretion of potassium increased by 20.6 mmol/ 24hr ($p<0.001$)		
He F *et al*, 2010	RCT	42	18-75 years	30M & 12F	1398 mg K (as K bicarbonate) or 1298 mg K (as K chloride) or placebo daily	Not reported	4 weeks	Placebo (Mean ± SD) Office BP SBP (mmHg): 145 ± 15 DBP (mmHg): 91 ± 9 Ambulatory BP (mmHg)	NS differences in office blood pressure among the 3 treatment periods, and only 24 hr & daytime	High

Reference	Study type	n	Age	Sex	Intervention and control or exposure	Dietary intake estimation	Follow up time	Results: effect, mean, SD, RR/OR/HR confidence interval, etc	Overall results	Risk of bias
								24 hr SBP: 142 ± 10 24 hr DBP: 88 ± 9 Day SBP: 148 ± 10 Day DBP: 93 ± 11 Night SBP: 135 ± 11 Night DBP: 82 ± 9 Plasma Measurements Na (mmol/l): 139 ± 2 K (mmol/l): 4.4 ± 0.3 Renin activity (ng/ml/hr; median): 0.12 (IQR 0.10-0.34) Aldosterone (pmol/l): 353 ± 165 βCTX (μg/l): 0.35 ± 0.13 <u>K chloride</u> Office BP SBP (mmHg): 142 ± 11 DBP (mmHg): 90 ± 9 Ambulatory BP (mmHg) 24 hr SBP: 139 ± 9 24 hr DBP: 87 ± 8 Day SBP: 146 ± 10 Day DBP: 92 ± 10 Night SBP: 133 ± 10 Night DBP: 81 ± 9 Plasma Measurements Na (mmol/l): 139 ± 2	SBP were slightly lower with potassium chloride. Compared with placebo, both K chloride & K bicarbonate significantly improved endothelial function as measured by brachial artery flow-mediated dilatation, increased arterial compliance as assessed by carotid-femoral pulse wave velocity, decreased left ventricular mass, & improved left ventricular diastolic function. NS difference between the 2 potassium salts in these	

Reference	Study type	n	Age	Sex	Intervention and control or exposure	Dietary intake estimation	Follow up time	Results: effect, mean, SD, RR/OR/HR confidence interval, etc	Overall results	Risk of bias
								K (mmol/l): 4.6 ± 0.2 Renin activity (ng/ml/hr; median): 0.17 (IQR 0.10- 0.32) Aldosterone (pmol/l): 387 ± 152 βCTX (µg/l): 0.34 ± 0.14 <u>K bicarbonate</u> Office BP SBP (mmHg): 144 ± 13 DBP (mmHg): 90 ± 9 Ambulatory BP (mmHg) 24 hr SBP: 142 ± 11 24 hr DBP: 89 ± 9 Day SBP: 149 ± 11 Day DBP: 95 ± 10 Night SBP: 135 ± 12 Night DBP: 82 ± 10 Plasma Measurements Na (mmol/l): 139 ± 2 K (mmol/l): 4.4 ± 0.3 Renin activity (ng/ml/hr; median): 0.17 (IQR 0.10-0.43) Aldosterone (pmol/l): 371 ± 141 βCTX (µg/l): 0.32 ±	measurements. The study also showed that K chloride reduced 24-hour urinary albumin and albumin:creatinine ratio, and K bicarbonate decreased 24 hr urinary Ca, Ca:creatinine ratio, and βCTX significantly	

Reference	Study type	n	Age	Sex	Intervention and control or exposure	Dietary intake estimation	Follow up time	Results: effect, mean, SD, RR/OR/HR confidence interval, etc	Overall results	Risk of bias
								0.11		
Naismith D *et al*, 2003	RCT	59 completed	25-65 years	36M & 26F	487 mg/d K (as 24 mmol KCl) or placebo	4 day dietary records & 3-day dietary checklist (list of fruit, vegetables and other food)	6 weeks	<u>Mean difference in BP (change at week 6 from baseline; mean ± SEM, 95% CI) for KCl group:</u> SBP: -6.22 ± 1.07; -8.40, -4.04 (p=0.001 from zero) DBP: -4.02 ± 0.87; -5.80, -2.23 (p=0.001 from zero) MAP: -4.91 ± 0.80; -6.54, -3.30 (p=0.001 from zero) <u>Mean difference in BP (change at 6 weeks from baseline; mean ± SEM, 95% CI) for placebo group:</u> SBP: 1.38 ± 0.95; -0.57, 3.33 (NS) DBP: 2.45 ± 0.73; 0.96, 3.94 (p=0.002 from zero) MAP: 2.09 ± 0.70; 0.68, 3.51 (p=0.005 from zero)	Urinary K could not be compared before and after supplementation as only baseline data was presented. Baseline urinary K was the same in both the K chloride and placebo groups. In the K group, MAP, SBP and DBP decreased significantly at 6 weeks compared to baseline. In the placebo group, MAP had increased significantly by 6 weeks compared to baseline. Compared with the placebo group, the K supplementation	High

Reference	Study type	n	Age	Sex	Intervention and control or exposure	Dietary intake estimation	Follow up time	Results: effect, mean, SD, RR/OR/HR confidence interval, etc	Overall results	Risk of bias
								Comparison of mean changes in BP between K and placebo groups (mean; 95% CI): SBP: -7.60; -10.46, -4.73(p=0.001 from zero) DBP: -6.46; -8.74, -4.19 (p=0.001 from zero) MAP: -7.01; -9.12, -4.89 (p=0.001 from zero)	group experienced a marked and significant decrease in SBP, DBP and thus MAP	
Sacks F *et al*, 1998	RCT	290	39 ± 5 years (Mean ± SD)	F	811 mg/d K (as 40 mmol KCl) or placebo	FFQ	16 weeks	Dietary K intake significantly increased compared to placebo in group 1 (K supplement; p<0.01) Urinary K excretion significantly increased from baseline to end in the K supplementation group, and was significantly different to placebo. 24 hr ambulatory BP change was significantly different to the change in the placebo group in the K supplemented group only (p=0.02,	Supplementation with potassium chloride at 40 mmol/d increased dietary K intake over 16 weeks. Ambulatory 24 hr BP was significantly lowered in normotensive individuals who received the K supplement .	High

Reference	Study type	n	Age	Sex	Intervention and control or exposure	Dietary intake estimation	Follow up time	Results: effect, mean, SD, RR/OR/HR confidence interval, etc	Overall results	Risk of bias
								systolic; p=0.01 diastolic). BP 24 hr ambulatory BP: K group average change from baseline, treatment-v-placebo: systolic BP: -2.0 (-3.7, -0.3) p=0.02 Diastolic BP: -1.7 (-3.0, -0.4) p=0.01		
Valdes G *et al*, 1991	RCT	24	50 ± 2.1 years (mean ± SEM)	13M & 11F	1298 mg/d K (as 64 mmol KCl) or placebo	Not reported	4 weeks	Serum K (mmol/l): Baseline: 3.8 ± 0.1; after placebo: 3.8 ± 0.1; after KCl: 4.1 ± 0.1 (p<0.001 for KCl compared to placebo) Urinary K (mmol/24 hr): Baseline: 57 ± 3; After placebo: 55 ± 4; After KCl: 123 ± 6 (p<0.001 for KCl compared to placebo) Supine BP (mmHg; systolic/diastolic): Baseline: 147/96 ± 3/1: After placebo: 145/92 ± 2/2; After KCl: 138/89 ± 3/2 (p<0.01 for	Urinary K & kallikrein excretion increased significantly following supplementation with KCl. Serum K significantly increased following supplementation with KCl. Urinary Kallikrein excretion with KCl correlated with urinary K levels at baseline,	High

Reference	Study type	n	Age	Sex	Intervention and control or exposure	Dietary intake estimation	Follow up time	Results: effect, mean, SD, RR/OR/HR confidence interval, etc	Overall results	Risk of bias
								systolic BP; KCl compared to placebo; p<0.05 for diastolic BP; baseline-v-placebo) Standing BP (mmHg; systolic/diastolic): Baseline: 148/101 ± 3/2; After placebo: 143/98 ± 2/2; After KCl: 138/94 ± 3/2 (p<0.05 diastolic BP KCl compared to placebo; p<0.05 systolic and diastolic BP, baseline-v-placebo) Urinary Kallikrein (mU/24 hr): Baseline: 651 ± 107; After placebo: 692 ± 69; After KCl: 1052 ± 141 (p<0.001 KCl compared to placebo)	with placebo and with KCl supplementation (r=0.44, p=0.0001) Supine DBP and standing SBP and DBP were significantly lower on placebo group than at baseline. After KCl supplementation, BP decreased significantly compared to the placebo group. Results from this study confirm the mild, mainly systolic antihypertensive effect of high K intake which has been reported by others	
Wilson D *et al*, 1996	RCT	40	13-15 years	22M & 18F	High K diet (providing 80 mmol/d K) or usual-diet control	Dietary records	4 weeks (1 week baseline; 3 weeks	*Data classified by "dippers" and "non-dippers" then sub-grouped by K diet and*	30 % of the adolescents were classified as dippers according	High

Reference	Study type	n	Age	Sex	Intervention and control or exposure	Dietary intake estimation	Follow up time	Results: effect, mean, SD, RR/OR/HR confidence interval, etc	Overall results	Risk of bias
					group.		dietary intervention phase).	*control diet:* Urinary K excretion in dippers (mean ± SD): K diet (n=15): baseline 38 ± 9; post-intervention 62 ± 19 control (n=13): baseline 38 ± 12; post-intervention 37 ± 8 Urinary K excretion in non-dippers (mean ± SD): K diet (n=5): baseline 40 ± 8; post-intervention 61 ± 15 control (n=7): baseline 39 ± 14; post-intervention 40 ± 11 *K intake changes from pre-to-post-intervention (mg) classified by "dippers" and "non dippers", subgrouped by intervention group:* Dippers: K diet: +1694 ± 1392; Control: +9 ± 640 Non-dippers: K diet: +2140 ± 912; Control: -37 ± 552 p<0.001 high K diet vs	to SBP classification. Urinary K significantly increased in the K diet group from baseline to post-intervention, regardless of dipper status (p<0.001), but remained unchanged in the control diet group from pre-to-post-intervention Subjects in the high K diet group showed a greater increase in K intake than did the subjects in the control diet group (p<0.001) Dippers had lower sleep SBP and DBP (and mean blood pressure)	

Reference	Study type	n	Age	Sex	Intervention and control or exposure	Dietary intake estimation	Follow up time	Results: effect, mean, SD, RR/OR/HR confidence interval, etc	Overall results	Risk of bias
								control diet *Blood Pressure-casual SBP/DBP (mmHg):* <u>Dippers:</u> K diet: baseline 109 ± 12/62 ± 7 post-intervention106 ± 10/60 ± 6 Control diet: baseline110 ± 8/63 ± 9 post-intervention108 ±8/59 ±7 <u>Non-dippers:</u> K diet: baseline 113 ± 8/62 ± 9 post-intervention 116 ± 10/61 ± 13 Control diet: baseline 111 ± 8/60 ± 9 post-intervention 109 ± 9/59 ± 9 *Blood pressure-Awake SBP/DBP:* <u>Dippers:</u> K diet: baseline 119 ± 8/ 67 ± 4 post-intervention 114 ± 11/64 ± 6 Control diet: baseline 119 ± 10/ 66 ± 5 post-intervention 117 ±	than non-dippers Dippers showed a decrease in SBP and DBP (and mean blood pressure) from baseline to post-intervention, whereas for non-dippers, BPs increased from baseline to post-intervention	

Reference	Study type	n	Age	Sex	Intervention and control or exposure	Dietary intake estimation	Follow up time	Results: effect, mean, SD, RR/OR/HR confidence interval, etc	Overall results	Risk of bias
								8/63 ± 5 <u>non-dippers:</u> K diet: baseline=115 ± 7/63 ± 7 post-intervention 124 ± 5/67 ± 4 Control diet: baseline 115 ± 7/63 ± 7 post-intervention 119 ± 7/66 ± 4 *Blood pressure: Asleep SBP/DBP:* <u>Dippers:</u> K diet: baseline 101 ± 8/55 ± 3 post-intervention 105 ± 11/56 ± 7 Control diet: baseline 99 ± 9/54 ± 4 post-intervention 103 ± 10/54 ± 6 <u>non-dippers:</u> K diet: baseline 110 ± 8/59 ± 5 post-intervention 110 ± 4/58 ± 4 control diet: baseline 111 ± 6/58 ± 3 post-intervention 112 ± 12/60 ± 6		

Table 4. Aldosterone and Renin

Reference	Study type	n	Age	Sex	Intervention and control or exposure	Dietary intake estimation	Follow up time	Results: effect, mean, SD, RR/OR/HR confidence interval, etc	Overall results	Risk of bias
Barden A *et al*, 1991	CT	37	Mean age (± SEM): K supplement group: 31.9 ± 1.7 years Placebo group: 32.2 ± 1.5 years	F	80 mmol/d K (as KCl) or placebo	Dietary habits questionnaire	4 days	Treatment effect (mean ± SEM; p value) Urinary 6-keto-PGF1α (ng/24 hr): +70.7 ± 34.4, p<0.05 Plasma ANP (pmol/l): -1.1 ± 0.4, p<0.01 Plasma Na+ (mmol/l): -0.8 ± 0.2, p<0.01 Plasma Renin Activity (ng/ml/hr): +0.1 ± 0.2, NS Plasma HCO3 (mmol/hr): -0.5 ± 0.3, NS Urinary K excretion (mmol/d; mean over the 4 day period ± SEM): Placebo: 53.4 ± 2.9; K supplement: 71.6 ± 4.3 (p<0.001) Change in Urinary Na excretion (mmol/d); mean over the 4 day period ± SEM: increased by 14.7 ± 4.5 mmol/d This natriuresis was observed in >75% of	Urinary 6-keto-PGF1α was significantly elevated at the end of 4 days K supplementation period (p<0.05) Significant increase in serum K equivalent to 0.29 ± 0.03 mmol/l (p<0.01) which was mirrored by a significant increase in plasma aldosterone (increased by average of 280 ± 68.5 pmol/l, p<0.01) ANP showed a small but significant decrease during 4 days K supplementation period	High

Reference	Study type	n	Age	Sex	Intervention and control or exposure	Dietary intake estimation	Follow up time	Results: effect, mean, SD, RR/OR/HR confidence interval, etc	Overall results	Risk of bias
								participants	(equivalent to 1.1 pmol/l, p<0.01). Change in ANP was negatively correlated with change in aldosterone (r=-0.47, p<0.01, n=29) Significant increases in 6-keto PGF1a after 4 days of K supplementation were significantly correlated to Na excretion, suggesting possible K-related natriuresis which may be mediated in part by prostaglandin	
Coruzzi P *et al*, 2001	RCT	11	23-46 years	8M & 3F	Isocaloric diet providing either 18 or 80 mmol/d K	Fixed diet	14 days	After 80 mmol/d K intake (mean ± SD) Serum Na (mmol/l): 143 ± 1 Serum K (mmol/l): 4.1 ±	After a 10 day period of low K intake serum K decreased (P < 0.001) by 0.9	High

Reference	Study type	n	Age	Sex	Intervention and control or exposure	Dietary intake estimation	Follow up time	Results: effect, mean, SD, RR/OR/HR confidence interval, etc	Overall results	Risk of bias
								0.05 Body weight (kg): 73.5 ± 3 PRA (ng/l/s): 0.47 ± 0.08 Plasma aldosterone (pmol/l): 1082 ± 161 Urinary Na (mmol/d): 199 ± 12 Urinary K (mmol/d): 67 ± 2 Urinary Ca (mmol/d): 4.7 ± 0.9 <u>After 18 mmol/d K intake (mean ± SD)</u> Serum Na (mmol/l): 142 ± 1 Serum K (mmol/l): 3.2 ± 0.1 Body weight (kg): 72.4 ± 3 PRA (ng/l/s): 0.25 ± 0.05 Plasma aldosterone (pmol/l): 710 ± 101 Urinary Na (mmol/d): 210 ± 14 Urinary K (mmol/d): 20 ± 1.5 Urinary Ca (mmol/d): 7.2 ± 1	mmol/l; NS changes in urinary Na and a marked increase in urinary Ca excretion ($P < 0.001$) were found during the 10-day low K intake. PRA ($P < 0.02$) and plasma aldosterone ($P < 0.04$) concentrations also decreased during low K intake in hypertensive patients. The magnitude of the natriuretic response exhibited by the whole hypertensive group during the two water immersion to the neck (WI) experiments was identical,	

Reference	Study type	n	Age	Sex	Intervention and control or exposure	Dietary intake estimation	Follow up time	Results: effect, mean, SD, RR/OR/HR confidence interval, etc	Overall results	Risk of bias
								p value Serum Na (mmol/l): NS Serum K (mmol/l): <0.001 Body weight (kg): <0.005 PRA (ng/l/s): <0.02 Plasma aldosterone (pmol/l): <0.04 Urinary Na (mmol/d): NS Urinary K (mmol/d): <0.0001 Urinary Ca (mmol/d): <0.001	however when Na excretion was expressed as a function of salt sensitivity, a greater % increase in Na excretion (r=0.69, p<0.02) was found in those individuals with a higher salt sensitivity index, undergoing central volume expantion by WI with concomitant K depletion than in those undergoing WI at normal potassium intake	
He F *et al*, 2005	RCT	14	Mean (± SD) 51 ± 9 years	11M & 3F	1952 mg K/d (as K chloride) or 1426 mg K/d (as K citrate)	Not reported	7 days	Baseline (mean ± SD) Plasma Na (mmol): 136 ± 2.4 K (mmol/l): 4.2 ± 0.3 Bicarbonate (mmol/l): 27 ± 0.3 Ca (mmol/l): 2.34 ± 0.10 P (mmol/l): 1.08 ± 1.66	Plasma aldosterone was significantly higher with both K chloride and K citrate compared with that at baseline.	High

Reference	Study type	n	Age	Sex	Intervention and control or exposure	Dietary intake estimation	Follow up time	Results: effect, mean, SD, RR/OR/HR confidence interval, etc	Overall results	Risk of bias
								Creatinine (µmol/l): 88 ± 19 Renin activity (ng/ml per hr): 0.49 ± 0.45 Aldosterone (pmol/l): 351 ± 144 Urine Na (mmol/24 hr): 161 ± 69 K (mmol/24 hr): ~80 Creatinine (mmol/24 hr): 16.1 ± 4.4 Ca (mmol/24 hr): 4.8 ± 2.2 pH: 6.23 ± 0.64 <u>K chloride</u> Plasma Na (mmol/l): 139 ± 2.1 K (mmol/l): 4.6 ± 0.3 Bicarbonate (mmol/l): 27 ± 2.9 Ca (mmol/l): 2.30 ± 0.11 P (mmol/l): 1.11 ± 1.03 Creatinine (µmol/l): 86 ± 18 Renin activity (ng/ml per hr): 0.58 ± 0.69 Aldosterone (pmol/l): 442 ± 165 Urine Na (mmol/24 hr): 139	Serum K was significantly higher after K supplementation than baseline, NS difference between the two forms. Plasma bicarbonate was significantly higher with K citrate compared with that with K chloride. With potassium citrate, there was a significant reduction in 24-hour urinary Ca & Ca/creatinine ratio, and a significant increase in urine pH, compared with that with K chloride or baseline. NS difference between K chloride & K	

Reference	Study type	n	Age	Sex	Intervention and control or exposure	Dietary intake estimation	Follow up time	Results: effect, mean, SD, RR/OR/HR confidence interval, etc	Overall results	Risk of bias
								±52 K (mmol/24 hr): ~170 Creatinine (mmol/24 hr): 15.4 ± 5.2 Ca (mmol/24 hr): 4.8 ± 2.8 pH: 5.89 ± 0.56 K citrate Plasma Na (mmol/l): 138 ± 2.0 K (mmol/l): 4.6 ± 0.3 Bicarbonate (mmol/l): 29 ± 2.2 Ca (mmol/l): 2.31 ± 0.13 P (mmol/l): 1.10 ± 0.17 Creatinine (µmol/l): 87 ± 16 Renin activity (ng/ml per hr): 0.54 ± 0.47 Aldosterone (pmol/l): 504 ± 166 Urine Na (mmol/24 hr): 145 ± 65 K (mmol/24 hr): ~165 Creatinine (mmol/24 hr): 16.0 ± 5.3 Ca (mmol/24 hr): 4.1 ± 2.6 pH: 7.40 ± 0.63	citrate or baseline in pulse rate, or body weight, or plasma Na, Cl^-, Ca, P, creatinine, or 24 hr urinary volume, Na, or creatinine excretion	

Reference	Study type	n	Age	Sex	Intervention and control or exposure	Dietary intake estimation	Follow up time	Results: effect, mean, SD, RR/OR/HR confidence interval, etc	Overall results	Risk of bias
Krishna G *et al*, 1991	RCT	12	23-58years	10M & 2F	Inital basal diet (60 mmol/d K & 150 mmol/d Na). followed by basal diet (16 mmol/d K & 120 mmol/d Na) plus placebo capsules or plus 80 mmol/d K as KCl	Not reported	15 days	<u>96 mmol/d K</u> Change in body weight (kg): -0.5 ± 0.4 Plasma Na (mmol/l):141 ± 1 Plasma K (mmol/l): 4.2 ± 0.1 Plasma Cl (mmol/l): 92 ± 1 Renin activity (ng/l per s): 0.38 ± 0.06 Aldosterone (pmol/l): 397 ± 47 Atrial natriuretic peptide (pg/ml): 34 ± 8 <u>16 mmol/d K</u> Change in body weight (kg): 0.2 ± 0.3 Plasma Na (mmol/l):140 ± 1 Plasma K (mmol/l):3.4 ± 0.1 Plasma Cl (mmol/l): 91 ± 2 Renin activity (ng/l per s): 0.25 ± 0.44 Aldosterone (pmol/l):105 ± 22 Atrial natriuretic peptide (pg/ml):58 ± 18	Plasma urinary K excretion was higher in the high K diet than the low K diet. Values are graphical (difficult to extract). By day 10 on high K diet, urinary excretion of K ~ 70 mmol/d Average daily urinary K excretion on the low K diet was 27 mmol, which exceeded daily intake by 11 mmol Urinary K depletion was accompanied by a decrease in plasma K concentration from pre-study levels in the low K diet phase	High

Reference	Study type	n	Age	Sex	Intervention and control or exposure	Dietary intake estimation	Follow up time	Results: effect, mean, SD, RR/OR/HR confidence interval, etc	Overall results	Risk of bias
								P value Change in body weight (kg): NS Plasma Na (mmol/l): NS Plasma K (mmol/l): <0.001 Plasma Cl (mmol/l): NS Renin activity (ng/l/s): 0.02 Aldosterone (pmol/l): <0.001 Atrial natriuretic peptide (pg/ml): NS Changes in K intake produced alterations in urinary Na excretion: on 96 mmol/d K diet, Na excretion was 110 ± 5 mmol/d over the 10d period; when on the 16mmol/d K diet, Na excretion was 83 ± 6 mmol/d over the 10d period (p<0.001)	Plasma renin activity and Aldosterone decreased during low K intake compared to the high intake (p<0.001)	
Lawton W *et al*, 1990	RCT	11	20-31 years	M	High Na, low K (9200 mg Na, 1170 mg K) diet or	Fixed diet	6 days	Normotensive (n=10) High K diet 24 hr urine	A low potassium diet is associated with disturbances	High

Reference	Study type	n	Age	Sex	Intervention and control or exposure	Dietary intake estimation	Follow up time	Results: effect, mean, SD, RR/OR/HR confidence interval, etc	Overall results	Risk of bias
					high Na, high K (9200 mg Na, 3900 mg K) diet daily			<u>Day 5</u> Na (mmol): 343 ± 20 K (mmol): 62 ± 4 Ca (mmol): 232 ± 33 Cl (mmol): 338 ± 18 Day 6 Serum Na (mmol/l): 139 ± 1 Serum K (mmol/l): 3.8 ± 0.04 Serum P (mmol/l): 3.4 ± 0.2 Na excretion (µmol/min): 280 ± 28 K excretion (µmol/min): 77 ± 10 Serum aldosterone (ng/dl): 3.6 ± 0.5 <u>Low K diet</u> 24 hr urine <u>Day 5</u> Na (mmol): 302 ± 21 K (mmol): 27 ± 2 Ca (mmol):326 ± 37 Cl (mmol): 358 ± 32 <u>Day 6, morning</u> Serum Na (mmol/l): 140 ± 1 Serum K (mmol/l): 3.5 ± 0.06 Serum P (mmol/l): 3.1 ±	in several electrolytes in borderline hypertensive subjects and normotensive subjects Serum Aldosterone levels were not different between normotensive and borderline hypertensive groups nor by diet Plasma renin activity during supine and standing procedures was not different between groups and was significantly lower after the potassium diet in the borderline hypertensive	

Reference	Study type	n	Age	Sex	Intervention and control or exposure	Dietary intake estimation	Follow up time	Results: effect, mean, SD, RR/OR/HR confidence interval, etc	Overall results	Risk of bias
								0.1 Na excretion (µmol/min): 390 ± 30 K excretion (µmol/min): 43 ± 4 Serum aldosterone (ng/dl): 2.9 ± 0.3 <u>Borderline Hypertensives (n=11)</u> <u>High K diet</u> 24 hr urine <u>Day 5</u> Na (mmol): 324 ± 20 K (mmol): 76 ± 4 Ca (mmol): 308 ± 36 Cl (mmol): 346 ± 31 <u>Day 6</u> Serum Na (mmol/l): 138 ± 1 Serum K (mmol/l): 3.8 ± 0.06 Serum P (mmol/l): 3.6 ± 0.1 Na excretion (µmol/min): 326 ± 24 K excretion (µmol/min): 2.4 ± 0.2 Serum aldosterone (ng/dl): 4.7 ± 0.4 <u>Low K diet</u> 24 hr urine	group in both positions (supine and standing) and was significantly lower during standing position in the normotensive group	

Reference	Study type	n	Age	Sex	Intervention and control or exposure	Dietary intake estimation	Follow up time	Results: effect, mean, SD, RR/OR/HR confidence interval, etc	Overall results	Risk of bias
								Day 5 Na (mmol): 322 ± 17 K (mmol): 30 ± 3 Ca (mmol): 353 ± 20 Cl (mmol): 303 ± 26 Day 6 Serum Na (mmol/l): 139 ± 1 Serum K (mmol/l): 3.6 ± 0.07 Serum P (mmol/l): 3.2 ± 0.1 Na excretion (µmol/min): 364 ± 29 K excretion (µmol/min): 40 ± 5 Serum aldosterone (ng/dl): 4.5 ± 0.7		

Table 5. Bone health

Reference	Study type	n	Age	Sex	Intervention and control or exposure	Dietary intake estimation	Follow up time	Results: effect, mean, SD, RR/OR/HR confidence interval, etc	Overall results	Risk of bias
Dawson-Hughes B *et al*, 2009	RCT	162	> 50 years	70M & 92F	1474 mg/d K as $KHCO_3$, 1369mg/d K as KCl, 67.5 mmol/d $NaHCO_3$, or placebo	FFQ	84 days	Urinary NTx/Creatinine change nmol/mmol): Placebo group ~+0.5 KCl ~-0.25 $KHCO_3$ ~-6.5 $NaHCO_3$ ~ -3.5 Data estimated from graph showing mean 3 month change in urinary NTx/Creatinine by treatment group adjusted for gender and baseline	The biochemical bone resorption marker, NTx/Creatinine, declined in the groups containing bicarbonate, but not in the non-bicarbonate groups. The change was significantly different between $KHCO_3$ and KCl treatment groups (p=0.015)	High
Karp H *et al*, 2009	RCT	12	22-30 years	F	2250 mg/d K (as K citrate) or placebo	Fixed diet	24 hours	K supplemented 24 hr urinary Ca:Creatinine (mmol/mmol): ~0.1 24 hr urinary Pi:Creatinine (mmol/mmol): ~1.6 24 hr urinary pH: ~7.5 24 hr urinary NTx: Creatinine (nmol BCE/mmol Creatinine):	K citrate supplementation, at least acutely, may decrease urinary Ca excretion and reduce bone resorption even when the diet is not acidogenic, Ca intake is low,	High

Reference	Study type	n	Age	Sex	Intervention and control or exposure	Dietary intake estimation	Follow up time	Results: effect, mean, SD, RR/OR/HR confidence interval, etc	Overall results	Risk of bias
								~16.2 <u>Placebo</u> 24 hr urinary Ca:Creatinine (mmol/mmol): ~0.14 24 hr urinary Pi:Creatinine (mmol/mmol): ~1.8 24 hr urinary pH: ~6.2 24 hr urinary NTx: Creatinine (nmol BCE/mmol Creatinine): ~22.3 Urinary Ca was lower in K citrate session than placebo (p=0.004). K citrate decreased serum Pi concentration compared to placebo (p=0.012). K citrate increased serum K significantly at 10:00 hrs (p=0.016), but not at other sampling times when compared to placebo. K citrate decreased urinary NTx (p=0.045) compared to placebo	and K intake is at recommended level	

Reference	Study type	n	Age	Sex	Intervention and control or exposure	Dietary intake estimation	Follow up time	Results: effect, mean, SD, RR/OR/HR confidence interval, etc	Overall results	Risk of bias
								K citrate had no significant effect on serum iCa, urinary Pi, serum PTH & serum bone specific alkaline phosphate (BALP) compared to placebo		
Macdonald H *et al*, 2008	RCT	276	55-65 years	F	K citrate low dose (18.5 mEq/d), K citrate high dose (55.5 mEq/d), Placebo capsule, or Fruit and vegetables dietary intervention group (300 g additional fruit and vegetables)	Diet record & 3-day dietary checklist (list of fruit, vegetables and other food)	24 months	Changes in urinary K excretion from baseline (mmol; mean ± SD): 3 months High K group: 38.1 ± 14.4 Low K group: 10.6 ± 26.3 Diet group: 15.1 ± 20.4 Placebo group: 4.1 ± 20.4 p=0.002 for high K group compared with other treatment groups 12 months High K group: 43.0 ± 18.2 Low K group: 11.6 ± 20.3 Diet group: 2.7 ± 28.6 Placebo group: -2.5 ±	There was a significant difference in meal daily K excretion between treatment groups at all follow-up time points Two years K citrate does not reduce bone turnover or increase BMD in healthy postmenopausal women	Moderate

Reference	Study type	n	Age	Sex	Intervention and control or exposure	Dietary intake estimation	Follow up time	Results: effect, mean, SD, RR/OR/HR confidence interval, etc	Overall results	Risk of bias
								39.6 p=0.001 for high K group compared with other treatment groups <u>24 months</u> High K group: 42.7 ± 29.9 Low K group: 8.3 ± 28.5 Diet group: 5.8 ± 27.9 Placebo group: -4.4 ± 27.4 p<0.001 for high K group compared with other treatment groups NS change in BMD for any groups across all follow up time points. NS changes in P1NP, ßCTX or fDPD/creatinine ratio at each follow-up time point. NS change in BMD for any groups across all follow up time points. NS changes in P1NP, ßCTX or		

Reference	Study type	n	Age	Sex	Intervention and control or exposure	Dietary intake estimation	Follow up time	Results: effect, mean, SD, RR/OR/HR confidence interval, etc	Overall results	Risk of bias
								fDPD/creatinine ratio at each follow-up time point NS difference in baseline dietary K intake between the 4 intervention groups		
Sebastian A *et al*, 1994	Balance study	18	51-77 years	F	Controlled diet (mean Ca: 652 mg; P: 871 mg; K: 59 mmol; Na: 119 mmol) followed by KHCO3 (60-120 mmol/d/60 kg bodyweight)	Duplicate diet	66 days	Change in Ca parameters during-v-before K supplementation phase (mg/d/60 kg): Stool: +8 ± 73 Urine: -64 ± 19 (p<0.001) Balance: +56 ± 76 (p<0.01) Change in K parameters during-v-before K supplementation phase (mg/d/60 kg): Stool: +2 ± 3 (p<0.001) Urine: +66 ± 26 (p<0.001) Balance: +11 ± 4 (p<0.001) Change in plasma K during-v-before K supplementation (mmol/l): +0.13 ± 0.12	Reduction in urinary hydroxyproline excretion in association with increases in serum osteocalcin concentrations in response to $KHCO_3$ supplementation suggests that administration of $KHCO_3$ appears to reduce the rate of bone resorption and increase the rate of bone formation, and may attenuate or	High

Reference	Study type	n	Age	Sex	Intervention and control or exposure	Dietary intake estimation	Follow up time	Results: effect, mean, SD, RR/OR/HR confidence interval, etc	Overall results	Risk of bias
								(p<0.001) Change in serum parameters during-v-before K supplementation: Total Ca (mg/dl): +0.08 ± 0.11 (p<0.02) 1,25-(OH2)-D (pg/ml): -1 ± 3.70 (NS) PTH (pg/ml): +2 ± 2.94 (p<0.02) osteocalcin (pg/ml): +0.6 ± 0.48 (p<0.001) Change in Ca parameters after-v-during K supplementation phase (mg/d/60 kg): Stool: -40 ± 84 Urine: +56 ± 23 (p<0.001) Balance: -12 ± 88 Change in K parameters after-v-during K supplementation phase (mg/d/60 kg): Stool: -2 ± 2 (p<0.001) Urine: -67 ± 26 (p<0.001) Balance: -11 ± 5 (p<0.001) Change in plasma K	reverse the loss of bone mass which occurs over the long term in post-menopausal women	

Reference	Study type	n	Age	Sex	Intervention and control or exposure	Dietary intake estimation	Follow up time	Results: effect, mean, SD, RR/OR/HR confidence interval, etc	Overall results	Risk of bias
								after-v-during K supplementation (mmol/l): -0.16 ± 0.15 (p<0.001) Changes in serum parameters after-v-during K supplementation: Total Ca (mg/dl): -0.08 ± 0.10 (p<0.02) 1,25-(OH_2)-D (pg/ml): +3 ± 3.51 (p<0.001) PTH (pg/ml): +0 ± 3.65 (NS) osteocalcin (ng/ml): -0.4 ± 0.49 (p<0.001) Change in urinary hydroxyproline excretion: Before: 28.9 ± 12.3 mg/d During: 26.7 ± 10.8 mg/d (p=0.05) Change in net renal acid excretion: before: 70.9 ± 10.1 mmol/d/60 kg; During: 12.8 ± 21.8 mmol/d/ 60kg		

Reference	Study type	n	Age	Sex	Intervention and control or exposure	Dietary intake estimation	Follow up time	Results: effect, mean, SD, RR/OR/HR confidence interval, etc	Overall results	Risk of bias
Sellmeyer D *et al*, 2002	RCT	52	placebo: 63 ± 8 years K citrate: 65 ± 8 years	F	Low Na diet (2000 mg/d) followed by High Na diet (5175 mg/d) plus 1334 mg K (as K citrate) or placebo daily	24 hr diet recall	7 weeks	<u>Change from low salt to high salt + K citrate (n=26; mean ± SE)</u> Urine Ca (mg/d): -8 ± 14 Urine NTx (nMBCE/mmol creatinine): 2.0 ± 1.7 Serum Ca (mg/dl): 0.05 ± 0.05 Osteocalcin (ng/ml): -0.22 ± 0.23 Fasting PTH (pg/ml): -0.74 ± 1.8 cAMP (nmol/l): 106.7 ± 135.6 Urine potassium (mEq/d): 72 ± 5 Net acid excretion (mEq/d): 60 ± 5 <u>Change from low salt to high salt + placebo (n=26; mean ± SE)</u> Urine Ca (mg/d): 42 ± 2 Urine NTx (nMBCE/mmol creatinine): 6.4 ± 1.4 Serum Ca (mg/dl): 0.04 ± 0.06 Osteocalcin (ng/ml): -0.57 ± 0.21 Fasting PTH (pg/ml):	The addition of oral K citrate to a high salt diet prevented the increased excretion of urine Ca and the bone resorption marker caused by high salt intake	Moderate

Reference	Study type	n	Age	Sex	Intervention and control or exposure	Dietary intake estimation	Follow up time	Results: effect, mean, SD, RR/OR/HR confidence interval, etc	Overall results	Risk of bias
								0.97 ± 1.5 cAMP (nmol/l): 1422 ± 99.2 Urine potassium (mEq/d): 2 ± 3 Net acid excretion (mEq/d): 3±3 <u>Change from low salt to high salt + placebo (p values)</u> Urine Ca (mg/d): 0.008 Urine NTx (nMBCE/mmol creatinine): 0.049 Serum Ca (mg/dl): NS Osteocalcin (ng/ml): NS Fasting PTH (pg/ml): NS cAMP (nmol/l): NS Urine potassium (mEq/d): <0.001 Net acid excretion (mEq/d): <0.001		
Sinaiko A *et al*, 1993	RCT	210	KCl group: 13.3 ± 0.1 years placebo group:	105M & 105F	low-sodium diet, KCl supplement (1 mmol/kg body weight, max 80 mmol/24 hr), or placebo capsule	Not reported	3 years	Mean rate of increase in BP is the Group mean rate of increase ("slope") over the 3 year intervention All boys groups had positive slope	Urinary 24hr K excretion was significantly increased in both boys and girls Systolic blood	High

Reference	Study type	n	Age	Sex	Intervention and control or exposure	Dietary intake estimation	Follow up time	Results: effect, mean, SD, RR/OR/HR confidence interval, etc	Overall results	Risk of bias
			13.4 ± 0.1 years (mean ± SD)					significantly different to zero, with no significant difference between the groups. For girls, the placebo group was a positive slope, significantly different to zero. The KCl group was slightly positive, but not significantly different to zero. Difference in systolic BP between girls and boys in placebo groups not significantly different. Difference in systolic BP between girls and boys in the KCl group significantly different ($p<0.01$)	pressure was affected by KCl intervention differently in girls than boys. In girls, systolic blood pressure over the 3 years significantly increased (as measured by "slope", which is the mean rate of increase) in the placebo group only. In girls, the rate of increase in BP over 3 years was lower in the K intervention group. In boys, there was no difference on the rate of increase in BP with K intervention	

Table 6. Diabetes

Reference	Study type	n	Age	Sex	Intervention and control or exposure	Dietary intake estimation	Follow up time	Results: effect, mean, SD, RR/OR/HR confidence interval, etc	Overall results	Risk of bias
Chatterjee R *et al*, 2011	Cohort study	12209	45-64 years	3847M & 8362F	Serum K status	Not reported	9 years	Baseline Serum K levels (mmol/l; mg/l) Blacks: 4.18 ± 0.45 (681 ± 17.55) Whites: 4.49 ± 0.43 (175.11 ± 16.77) p<0.01 Adjusted RH (relative hazards) 95%CI, for incident diabetes for each category of serum K in African Americans and whites (compared to those with serum K of 5-5.5mEq/l): <4 mEq/l serum K: African American: 2.28 (1.21, 4.28); Whites: 1.53 (1.14, 2.05) 4-4.4 mEq/l serum K: African American: 1.97 (1.06, 3.65); Whites: 1.49 (1.19, 1.87) 4.5-4.9 mEq/l serum K: African American: 1.85 (0.99, 3.47); Whites: 1.27 (1.02, 1.58)	Mean serum K concentrations were significantly lower in blacks than in whites (p<0.01) There was a graded inverse relationship between serum K and incident diabetes in both African Americans and Whites. The serum K-race interaction was not statistically significant. When average serum K from baseline and 3 year follow up was used in analyses, average serum K	High

Reference	Study type	n	Age	Sex	Intervention and control or exposure	Dietary intake estimation	Follow up time	Results: effect, mean, SD, RR/OR/HR confidence interval, etc	Overall results	Risk of bias
									was significantly associated with diabetes risk (p=0.0003) and was more strongly associated with African Americans then in whites Thus, low normal serum K is associated with a greater risk of incident diabetes and with greater risk hazards in African Americans than in whites	

Table 7. Resting metabolic rate

Reference	Study type	n	Age	Sex	Intervention and control or exposure	Dietary intake estimation	Follow up time	Results: effect, mean, SD, RR/OR/HR confidence interval, etc	Overall results	Risk of bias
Deriaz O *et al*, 1991	CT	8	26 ± 2 years (mean ± SD)	M	High K diet (6357 mg/d K) or low K diet (2691 mg/d K) followed by single 50 mmol KCl (low K diet) or placebo (high K diet) on day 5 n=8 crossover study	Fixed diet	5 days	<u>High K diet (mean ± SE)</u> Dietary K intake (mmol/d): 163 ± 9 Energy intake (MJ/d): 12.1± 0.7 Urinary K excretion (mmol/d): 119 ± 14mmol/d Urinary Na (mmol/d): 237 ± 24 Resting energy expenditure-post placebo (MJ/d): 7.6 ± 0.3 Serum K (mmol/l):3.9 ± 0.1 Serum Na (mmol/l): 140.2 ± 0.4 <u>Low K diet</u> Dietary K intake (mmol/d): 69 ± 2 Energy intake (MJ/d): 12.4 ± 1.8 Urinary K excretion (mmol/d): 50 ± 3 Urinary Na excretion (mmol/d): 185 ± 15 Resting energy expenditure-post KCl (MJ/d): 7.6 ± 0.3 Serum K (mmol/l):4.4 ± 0.1	High & low K diets for 4 days did not change RMR. However, variation in RMR was significantly correlated with changes in serum K	High

Reference	Study type	n	Age	Sex	Intervention and control or exposure	Dietary intake estimation	Follow up time	Results: effect, mean, SD, RR/OR/HR confidence interval, etc	Overall results	Risk of bias
								Serum Na (mmol/l): 141.3 ± 0.2 <u>Difference between low and high K diets (p value):</u> Dietary K intake: p<0.0001 Energy intake: NS Urinary K excretion (mmol/d): p<0.05 Urinary Na excretion: NS Serum K: p<0.05 Serum Na: NS There was a high correlation between K intake and urinary K output (r=0.94, p<0.001) Significant relationship was found between chronic serum K changes and RMR changes, both during baseline periods (r=0.74, p<0.05) and post-KCl and baseline periods (r=0.74, P<0.05). No relationship between acute serum K changes and RMR (r=0.22) Significant relationship was found between chronic		

Reference	Study type	n	Age	Sex	Intervention and control or exposure	Dietary intake estimation	Follow up time	Results: effect, mean, SD, RR/OR/HR confidence interval, etc	Overall results	Risk of bias
								serum K changes and RMR during post-KCl and post-placebo periods (r=0.74, p<0.05)		

Table 8. Sleep

Reference	Study type	n	Age	Sex	Intervention and control or exposure	Dietary intake estimation	Follow up time	Results: effect, mean, SD, RR/OR/HR confidence interval, etc	Overall results	Risk of bias
Drennan MD *et al*, 1991	RCT	9	18-33years	M	Low K diet (1590 mg) plus 1947 mg K (as K chloride) or placebo daily	Not reported	7 days	<u>K vs placebo</u> <u>Actigraph (n=6)</u> Sleep onset: NS Sleep offset: NS Sleep interval (min): NS Sleep efficiency (%): p<0.05 (↑K group) Sleep latency (min): NS WASO (min): p<0.05 (↓ K group) Total sleep (min): NS <u>Sleep log (n=9)</u> Sleep onset: p<0.001 (↓ K group) Sleep offset: NS Sleep interval (min): p< 0.001 (↓ K group) Serum potassium (mEq/l): p<0.05 (↑K group)	K significantly delayed sleep-log-identified Bedtime (p<0.001) & reduced Sleep interval for sleep log (p<0.01). K significantly increased Sleep efficiency (p<0.05) due to a reduction in actigraph WASO (p<0.05)	High

Table 9. Breast milk concentration

Reference	n (samples)	Country	Maternal K intake (mg/day; mean ± SD)	Stage of lactation	K level (mg/l) mean ± SD	median ± SD	Risk of bias
Holt C, 1993	4 (28)	UK	Not reported	5-16 weeks	592.8 ± 85.8		High
Naqvi H and Baseer A, 2001	25 (100)	Pakistan	Not reported	Not reported anovulatory ovulatory	 443.82 ± 13.26 422.76 ± 10.14		High
Parr R *et al*, 1991	330	Guatemala Hungary Nigeria Philippines Sweden Zaire	Not reported	3 months		487 ± 10 554 ± 9 410 ± 42 469 ± 11 548 ± 19 511 ± 10	High
Qian J *et al*, 2009	120 (120)	China	median (interquartile range): Group 1: 2602 (2436, 2943) Group 2: 2724 (2513, 2964) Group 3: 2704 (2437, 3198) Group 4: 2320 (2129, 2685)	8-10 days	median (interquartile range): Group 1: 620 (530, 690) Group 2: 610 (560, 680) Group 3: 630 (590, 680) Group 4: 470 (430, 480)		High
Rakicioğlu N *et al*, 2006	21 (42)	Turkey	During Ramadan: 1908.2 ±752.1 After Ramadan: 1942.8 ±792.4	2-5 months During Ramadan: After Ramadan:	 239.6 ± 52.7 322.7 ± 59.6		High
Sinchai W *et al*, 1995	110	Thailand	Not reported	6 weeks (baseline data)		Mean range: 534.3-549.9	High

Reference	n (samples)	Country	Maternal K intake (mg/day; mean ± SD)	Stage of lactation	K level (mg/l) mean ± SD	median ± SD	Risk of bias
Wack R *et al*, 1997	30 (140)	USA	Not reported	0-60 days 61-120 days 121-180 days 181-240 days 241-300 days 301-360 days >360 days	585 ± 124 490 ± 85 485 ± 66 473 ± 63 470 ± 72 445 ± 53 461 ± 89		High
Yamawaki N *et al*, 2005	(1197)	Japan	Not reported	1-5 days 6-10 days 11-20 days 21-89 days 90-180 days 181-365 days Summer Winter	723 ± 127 709 ± 228 639 ± 104 466 ± 83 434 ± 103 432 ± 70 455 ± 119 485 ± 122		High
Yurdakok M *et al*, 1991	19 (19)	Turkey	Not reported	1-7 days	629.07 ± 145.47		High

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SCIENTIFIC REPORT submitted to EFSA

Literature search and review related to specific preparatory work in the establishment of Dietary Reference Values

Preparation of an evidence report identifying health outcomes upon which Dietary Reference Values could potentially be based for magnesium, potassium and fluoride [4]

Fluoride

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Abstract

The objective of this systematic search and review was to identify the scientific data from January 1990 to March 2011 upon which Dietary Reference Values (DRVs) may potentially be based for fluoride.

Articles were identified using Medline, EMBASE (both on Ovid SP) and the Cochrane Library CENTRAL databases. Duplicate references were removed and additional studies identified by checking reference lists of pertinent reviews, included studies and current reports on DRVs. A total of 2181 articles were screened on the basis of title and abstract, resulting in 254 articles retrieved for full-text assessment, and the final inclusion of 43 studies (45 articles) for this review.

Bone and tooth health were included as health endpoints. Bone health was addressed by one systematic review (SR) and one nested case-control study, both finding little evidence of a protective role for fluoride. For tooth health, eight studies (5 SRs and 3 intervention trials) were included, largely focusing on caries risk reduction in children. There was evidence to support a beneficial role for fluoride in caries risk reduction, although systematic reviews identified by the search acknowledged that there were relatively few trials of good quality in this area. One randomised controlled trial reported on the influence of fluoride on leptin levels and found no significant change following fluoride supplementation.

The majority of original studies included (16) focused on the metabolism of fluoride, particularly absorption from tablets or aqueous solutions, generally agreeing that concurrent administration of a meal reduced fluoride bioavailability. However, studies addressing meal influences were assessed as being at high risk of bias.

Overall, there was a lack of high quality evidence upon which DRVs may potentially be based for fluoride.

Summary

This systematic search and review was carried out preparatory to work by EFSA to establish Dietary Reference Values (DRVs) for magnesium, potassium and fluoride, Lot 3 from the open call for tender CFT/EFSA/NDA/03. This report summarises the findings on fluoride.

The literature was comprehensively searched from January 1990 to March 2011 for studies in the English language. The search focused on primary research in humans concerning maintenance of functional competence and the prevention of clinical deficiency and chronic disease upon which DRVs may be based. Only studies reporting a quantitative relationship between i) intake and status; ii) intake and health; or iii) status and health were included (with the exception of studies reporting fluoride concentration in breast milk).

Articles were identified using Medline, EMBASE (both on Ovid SP) and the Cochrane Library CENTRAL databases. Complex search strategies using index and text terms, truncating and Boolean operators were developed and refined for each database. The search results were combined and imported into Endnote® (version X4, Thomson Reuters, New York) duplicate references were removed, resulting in 2174 references to screen. A further seven articles were identified by checking reference lists of pertinent reviews, included studies and current reports on DRVs. In total, 2181 articles were screened on the basis of title and abstract, resulting in 254 articles being retrieved for full text assessment, and the final inclusion of 45 articles, representing 43 studies, for this review which satisfied all inclusion criteria. For each study, data on design, methodology, results and validity were fully extracted into a Microsoft Excel® (Microsoft Corp, Seattle) database, and the key data summarised.

This review only reports on fluoride intake levels below the tolerable upper intake (1.5 mg/day for children aged 1-3 years, 7 mg/day for adolescents and adults ≥15 years), and only included forms of fluoride that are naturally present in foods, or approved by the EC for use in foods (potassium fluoride; sodium fluoride) or food supplements (calcium fluoride; potassium fluoride; sodium fluoride; sodium monofluorophosphate).

Studies which met the inclusion criteria were: 5 systematic reviews (SRs); 13 randomised controlled trials (RCTs) (14 articles); 12 non-randomised controlled trials (CTs); 1 balance study; 1 case-control (2 articles); 1 nested case-control; and 10 cross-sectional studies, although there is some overlap between these studies due to the inclusion of systematic reviews. Quality is reported for each article (45), rather than for each study (43), as the study endpoints differed in each publication. Applying the quality scheme adapted from the EURRECA Network of Excellence, articles were assessed as being at high risk of bias (37); moderate risk of bias (6) and low risk of bias (2).

Health endpoints included by this systematic review were tooth and bone health. Two studies relating to bone health were included (1 SR at moderate risk of bias [that included 3 other SRs and 3 cross-sectional studies]; plus an additional nested case-control at low risk of bias), neither of which found conclusive evidence for a beneficial role for fluoride. There were eight studies (9 articles) assessing tooth health (5 SRs [including between them 3 SRs; 18 RCTs; 1 cohort; 11 cross-sectional studies]; plus an additional 2 RCTs and 1 CT). These studies investigated caries risk reduction, with the exception of one trial which looked at the dentine and enamel content of fluoride. The articles were assessed as being at low (1); medium (4) and high risk of bias (4). The data suggest a protective role for fluoride in terms of the risk reduction in dental caries, although systematic reviews reported a lack of quality trials in this field that quantified total fluoride intake. One RCT assessed as being at high risk of bias reported on a biomarker (leptin) of disease outcome (obesity) and found no significant relationship with fluoride intake.

Included fluoride biokinetic studies were separated into studies focused on fluoride bioavailability and metabolism (16), and fluoride status (7). Serum and urinary fluoride concentrations were used as typical status markers. Absorption studies (in adult groups) were

in agreement that the administration of a meal within 30 minutes of a fluoride supplement reduced the peak fluoride concentration within the plasma and lengthened the time to reach peak levels. However, studies which reported on the influence of a meal on fluoride absorption were assessed as being at high risk of bias. Studies assessing urinary fluoride excretion generally agreed that fluoride intake was well correlated with 24 hour urinary excretion, although wide between- and within- subject variability was found.

One polymorphism case-control study (2 articles), assessed as being at high risk of bias, was included which explored polymorphisms of osteocalcin and collagen genes (HindIII; COL1A2 Rsa1l; COL1A2 PvII) but evidence was inconclusive.

There were nine cross-sectional studies which reported the concentration of fluoride in breast milk, which was found to vary widely. All but one study were assessed as being at high risk of bias.

Few studies were identified which met the study inclusion criteria and the majority were assessed as being at high risk of bias. Overall, there was a lack of high quality evidence upon which DRVs may potentially be based for fluoride. However, data was suggestive of a protective role for fluoride in the reduction of dental caries.

Key words: fluoride, systematic review, Dietary Reference Values (DRVs), dietary requirements, health outcomes, biomarkers, status, bioavailability

Introduction

This report focused on identifying information to inform the setting of Dietary Reference Values (DRVs) for fluoride. There is some debate over the essentiality of fluoride and no specific recommendations for intake were made by the Scientific Committee for Food (SCF, 1993). Despite limited evidence on which to base a dietary recommendation for fluoride, beneficial effects in the prevention of dental caries and improved bone mineral density have been reported (SCF, 1993; SCF, 2006). In some countries, these protective roles have led to the introduction of fluoridated water supplies or other products, such as fluoridated salt, milk or dental products including toothpaste, mouthwashes, gels and paints (SCF, 2006). These products are regularly consumed but individual use of fluoridated products varies considerably and is rarely reported thoroughly. As a result, obtaining accurate estimates of total daily fluoride intake is difficult and unreliable (Whitford, 1994).

This review only reports on fluoride forms present naturally in foods or those approved by the EC (EC Directive 2002/46/EC; EC Regulation No 1925/2006) for use in foods (potassium fluoride; sodium fluoride) or food supplements (calcium fluoride; potassium fluoride; sodium fluoride; sodium monofluorophosphate). Major fluoride food sources are marine fish, fluoridated salt, tea, water, water based beverages or foods reconstituted with fluoridated water e.g. soup or infant formulas. This review will concentrate on intakes below the Tolerable Upper Intake Level (UL), set at 1.5 mg/day for children aged 1-3 years and up to 7 mg/day for adolescents and adults ≥15 years (SCF, 2006).

Biomarkers for fluoride have been identified (SCF, 2006; Whitford, 1994), but are problematic and not well defined. Body fluids (saliva, plasma, and urine [the major route for excretion]) give some indication of short-term intake, although saliva concentration will also reflect fluoride exposure in the oral cavity. In contrast, nail and hair samples may provide information on longer term fluoride intake. Bone and dentine levels give an indication of cumulative life-time exposure, although measures are affected by uneven fluoride distribution and bone turnover. Enamel represents fluoride availability at the time of tooth formation, with only surface enamel reflecting recent fluoride levels in dentifrice, saliva and food (SCF, 2006; Whitford, 1994).

Both quality of dietary intake data and biomarkers used were carefully considered as part of the study inclusion criteria. The dose of fluoride consumed had to be accurately quantified (including exposure to dentifrice where applicable), or consumption reflected by a change in biomarker status. All biomarkers were included in this review, with the exception of salivary fluoride which was not considered to be a reliable biomarker of fluoride intake due to the influence of direct exposure to fluoride in the oral cavity.

Specific objectives and methodology

The purpose of this work was to collate the scientific data from which Dietary Reference Values for fluoride may be derived, building on existing advice of the Scientific Committee for Food Dietary Reference Values report of 1993.

In March 2011, the electronic searches were run following rigorous development and optimisation of the complex search strategy (which included indexing and text terms, truncation and Boolean operators). Fluoride specific search strategies are detailed in **Appendix A: Fluoride.**

Methods followed those described in the Materials and Methods section above. However following discussion within the review group regarding the strength of evidence for fluoride, and due to the particular difficulties in accurate determination of fluoride intake, the following exclusion criteria specific to fluoride were applied:

Data not relevant to doses below the UL set at 1.5 mg/day for children aged 1-3 years and up to 7 mg/day for adolescents and adults ≥15 years (except when investigating balance and bioavailability factors).

Studies using only saliva as a fluoride biomarker.

Studies without a suitable control and/ or baseline measure.

Cross-sectional studies not focused on intake and status, or cross-sectional studies with less than 100 participants (with the exception of studies reporting fluoride concentration in breast milk).

Balance studies without both urinary and faecal excretion measures.

Studies not accurately quantifying fluoride dose, nor measuring a change in biomarker status to reflect changes in fluoride consumption.

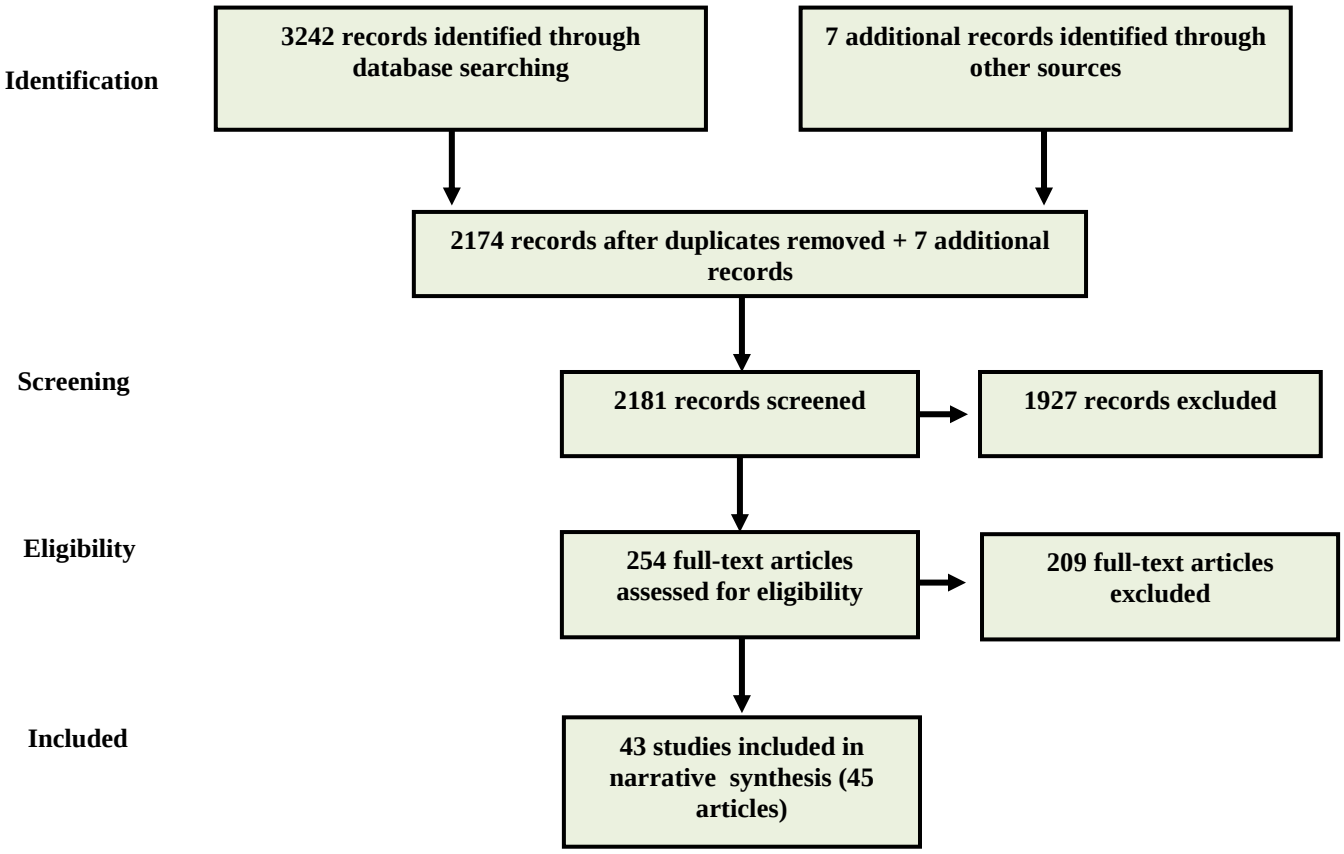
Short term biokinetic studies (≤24 hours) not including intake in a dietary form (foods, water, other beverages).

Studies using inappropriate methodology (insufficient detail to draw conclusions).

Results

A total of 3242 records were identified through database searching, duplicate references (1068) were removed, resulting in 2174 references to screen. A further seven articles were identified by checking reference lists of pertinent reviews, included studies and current reports on DRVs. In total, 2181 articles were screened on the basis of title and abstract, resulting in 254 articles retrieved for full text assessment, and the final inclusion of 43 studies (45 articles) for this review which satisfied all inclusion criteria. These results are summarised in the PRISMA flow chart (**Figure 1**; Moher *et al*, 2009):

Figure 1. PRISMA flow chart



The 43 studies included were classified as: systematic reviews (5); randomised controlled trials (13); non-randomised controlled trials (12); balance study (1); case-control (1); nested case-control (1); cross-sectional studies (10). These 43 studies were reported in 45 articles. The case-control study was published in two papers (Ba *et al*, 2009; Huang *et al*, 2008) assessing different polymorphisms of osteocalcin and collagen genes, but conducted in the same participant study group. Two randomised controlled trials assessing tooth health (Leverett *et al*, 1997; Sa Roriz Fonteles *et al*, 2005) started with the same group of participants (n=798). Leverett *et al* (1997) assessed caries risk reduction, whilst Sa Roriz Fonteles *et al* (2005) followed-up a random subsample of this population (n=185) to assess any changes in the fluoride content of enamel and dentine. The data have been presented separately for the 45 articles for ease of data interpretation and are grouped by endpoint in the following subsections: bone health; tooth health; biomarkers of disease; bioavailability and metabolism; status markers; polymorphisms; breast milk concentration. There was a degree of overlap between the included studies, particularly for the systematic reviews and this has been described in detail within the relevant subsections.

Health endpoints included in this systematic review were tooth and bone health. Two studies relating to bone health were included (1 SR at moderate risk of bias [that included 3 other SRs and 3 cross-sectional studies]; plus an additional nested case-control at low risk of bias). There were eight studies assessing tooth health, largely focused on caries risk reduction in children (5 SRs [including between them 3 SRs; 18 RCTs; 1 cohort; 11 cross-sectional studies]; plus an additional 2 RCTs and 1 CT). One randomised controlled trial reported on the response of a health biomarker (leptin), to supplemental fluoride intake. Higher leptin levels have been linked to a greater risk of obesity by contributing to changes in energy intake and metabolism (Oral and Ozbasar, 2002).

There were 16 studies focused on fluoride bioavailability and metabolism, all but one of these were conducted in adult subjects. Only one balance study was included and this study did not account for fluoride sweat loss. Furthermore, only three studies addressing fluoride metabolism were conducted over the longer-term (>48 hours). There were seven studies included measuring fluoride status marker changes following fluoride consumption, but the type of biomarkers used was limited. Urinary fluoride was used as the status marker in six studies, and nail fluoride was used in the one remaining study. Two polymorphism case-control studies were included which explored polymorphisms of osteocalcin and collagen genes (HindIII; COL1A2 Rsa1l; COL1A2 PvII) in children. There were nine cross-sectional studies reporting on the concentration of fluoride in breast milk, which were included due to their use in determining previous DRVs (IOM, 1997; NHMRC, 2006).

The included studies are summarised by primary endpoint, study type and population in **Table 1**. The study methodology, results and quality are described in detail in the sections below and summarised by endpoint in **Tables 2-8**.

Table 1. Summary of studies included [(a)]

Endpoint	Study type	Population
Bone health (2) (b)	SR (1) Nested case-control (1)	Any (1) Adult, female (1)
Tooth health (8) (b) (9 articles)	SR (5) RCT (2) CT (1)	Any (1) Adult, mixed (1) Children, mixed (6)
Biomarker of disease (leptin levels) (1)	RCT (1)	Adult, female (1)
Bioavailability and metabolism (16)	RCT (10) CT (5) Balance (1)	Adult, mixed (9) Adult, female (2) Adult, male (4) Infants (1)
Status markers (7)	CT (6) Cross-sectional (1)	Adult, female (2) Adult, male (1) Children, mixed (4)
Polymorphisms (1) (2 articles)	Case-control (1)	Children, mixed (1)
Breast milk concentration (9)	Cross-sectional (9)	Adult, female (9)

(a): Number of studies included for each endpoint, study type and population is given in brackets
(b): The NHMRC review (2007) covered both tooth and bone health and has been included in both sections

Validity

Quality assessment is normally completed for each included study, but in this instance each article (45) was assessed individually, rather than for each study (43), as endpoints for each publication differed. Articles were assessed as being at high risk of bias (37); moderate risk of bias (6) and low risk of bias (2). The number of studies assessed as being at a high or moderate risk of bias and key reasons for this assessment are summarised by study type below:

SR. *High risk of bias*: Did not adequately report study inclusion criteria, study assessment and data pooling (1).

Moderate risk of bias: Did not adequately report study assessment (1); study validity (1); or display study characteristics adequately (1).

RCT. *High risk of bias:* Non-randomised (12); Randomised but did not adequately report the method of randomisation, allocation concealment or blinding (12).

Moderate risk of bias: Did not adequately report background dietary exposure (2).

Balance. *High risk of bias:* Did not assess sweat loss (1).

Case-control. *High risk of bias:* Did not adequately report adjustment for confounders (2).

Cross-sectional. *High risk of bias:* Did not adequately assess fluoride intake (8); Did not adequately report adjustment for confounders or report if the sample was representative of the population (1).

Moderate risk of bias: Did not adequately report adjustment for confounders (1).

BONE HEALTH (TABLE 2)

Two studies relating to bone health were included (1 SR at moderate risk of bias [that included 3 other SRs and 3 cross-sectional studies]; plus an additional nested case-control at low risk of bias).

The case-control study (Feskanich *et al*, 1998) was nested within the Nurses' Health Study which aimed to determine if nail fluoride content could be used as an indicator of bone health. The data were equivocal and not dose-dependent, with higher levels of nail fluoride (>5.50 ppm) being associated with a slightly reduced risk of hip fracture, but with an increased risk of forearm fracture.

One comprehensive review conducted by the Australian National Health and Medical Research Council "A Systematic Review of the Efficacy and Safety of Fluoridation" was included (NHMRC, 2007). This review evaluated literature relating to tooth health (**Table 3**) in addition to bone health. The NHMRC review covered fluoridation delivery suitable for widespread public health interventions i.e. water, milk and salt (in addition to topical treatments). No individual studies of fluoridated water (public water supply or other water sources e.g. bore holes) met our study inclusion criteria, since total fluoride intake was not quantified; however, as a comprehensive systematic review, data from the NHMRC review have been summarised here (**Table 2**). The NHMRC review found few high quality trials relating to bone health, with three SRs and three cross-sectional studies of water fluoridation meeting the inclusion criteria (with no studies included for salt or milk fluoridation). The conclusion of this review was that there was little evidence for positive (or negative) associations between fluoride intake and bone mineral density and/or fracture risk. However, the NHMRC (2007) review recommended water fluoridation levels of between 0.6 and 1.1 ppm, in line with findings relating to caries prevention (**Table 3**).

TOOTH HEALTH (TABLE 3)

In total eight studies (9 articles) were identified which met the inclusion criteria and assessed tooth health (5 SRs, 2 RCTs, 1 CT). Some studies were interlinked and data were extracted for each article separately for ease of data interpretation. **Table 3** lists the characteristics and

results of these studies and also includes cross-referencing where studies are related. The five systematic reviews identified, included between them a total of: 3 SRs; 18 RCTs; 1 cohort; 11 cross-sectional studies. Any studies in these systematic reviews which met our inclusion criteria have been included separately in **Table 3**. This applied to two RCTs (out of the 18 included within the SRs), Leverett *et al* (1997) and Lin and Tsai (2000). Other trials included in the systematic reviews did not meet our study inclusion criteria (published prior to January 1990; non-English articles, abstract only; unsuitable control group; unsuitable fluoride form; or insufficient data on total fluoride intake- particularly for water fluoridation studies). Whilst SRs are deemed the highest level of evidence, it should be noted that as a number of their included studies did not meet our inclusion criteria, their findings need to be interpreted with this in mind. Two other additional articles were included: Mulyani and McIntyre (2002; CT); and Sa Roriz Fonteles *et al* (2005; RCT) which was a follow-up of the Leverett *et al* (1997) trial. The majority of the tooth health studies were conducted in children. These studies reported changes in the proportion of caries-free individuals and changes to caries status (most commonly, number of decayed, missing and filled teeth), with the exception of Sa Roriz Fonteles *et al* (2005) who reported on fluoride levels within dentine and enamel.

The five systematic reviews (only 1 assessed as being at high risk of bias) looked at a wide range of fluoride vehicles (milk, salt, water, supplements). For fluoridated milk (2.5-7.5 mg fluoride/l) and salt (intake levels not reported), systematic reviews agreed that there was insufficient studies of high quality evidence, although findings were supportive of caries risk reduction. Data for fluoride supplements (Espelid, 2009; Ismail and Hasson, 2008) were stronger with evidence for a reduction of caries in those supplemented with fluoride at doses $\leq$2 mg/day. Two of the included systematic reviews which addressed water fluoridation and tooth health were included since these reviews pooled data on water fluoridation.The most comprehensive was the NHMRC review (2007) which included data from previous systematic reviews in additional to individual studies. Griffin *et al* (2007) was the only systematic review included which was not already covered by the NHMRC review and is unusual in that only studies of adults with a mean age of $\geq$20 years were included. Both reviews found significant beneficial effects of fluoridation on caries prevention. Griffin *et al* (2007) found protective effects at fluoridation levels of 0.7-3.5 ppm in comparison with levels of 0.1-0.7 ppm, whilst the NHMRC (2007) review recommended fluoridation levels of between 0.6 and 1.1 ppm. Whilst these systematic reviews were of moderate quality, they report shortcomings in the quality of studies included in terms of study design and assessment of fluoride exposure. Additionally two Cochrane systematic reviews were identified relating to caries risk, but were at the protocol stage and results were not published at the time of writing. These studies are due to report on the efficacy of salt fluoridation (Gillespie *et al*, 2007) and fluoridated tablets, drops, lozenges and chewing gum (Tubert-Jeannin *et al*, 2009).

The RCT/ CTs included in addition to the systematic reviews in this review, were assessed as being at high risk of bias, with the exception of Leverett *et al* (1997), which was of moderate quality. This study and the follow-up study (Sa Roriz Fonteles *et al*, 2005) found no significant effect of pre-natal supplements (1 mg fluoride/day to mothers during pregnancy) in improving tooth health in children up to five years of age. The remaining two studies (0.25 mg

and 0.6 mg fluoride/day to children) did find a significant reduction in caries risk, although these studies were assessed as being at high risk of bias and had fewer participants per study group.

One cross-sectional study, measuring fluoride levels in breast milk and performing a dental examination in infants, was identified (Hossny *et al*, 2003). Infant fluoride serum levels were also measured in this trial. However, the sample size for infants exclusively fed with breast milk was too low (n<100) to meet the study inclusion criteria, and therefore only the breast milk data have been reported (**Table 8**), the study was also assessed as being at high risk of bias. Hossny *et al* (2003) found that fluoride levels in breast milk were not significantly correlated with infant fluoride serum concentrations, and serum concentrations in those with delayed primary dentition were comparable to those with normal dentition for age.

BIOMARKERS OF DISEASE (TABLE 4)

One randomised controlled trial reported on the influence of fluoride on plasma leptin levels. Leptin levels were higher in participants with a BMI ≥25. Long term administration of a relatively low dose of fluoride (3.3 mg fluoride/day) was not found to significantly influence leptin levels, although treatment resulted in a tendency to a reduced concentration. This study also noted that bone mineral density significantly increased by a small degree in the treatment group, but did not provide data on the analysis or any pertinent results. The study was assessed as being at a high risk of bias.

BIOAVAILABILITY AND METABOLISM (TABLE 5)

For ease of data interpretation the fluoride biokinetic trials included have been separated into those focused on fluoride bioavailability and metabolism (**Table 5**) and those focused on fluoride intake and status relationships (**Table 6**). Bioavailability and metabolism studies described here relate to the influence of different fluoride forms or nutrient interactions, which may affect fluoride bioavailability and retention. Commonly plasma and/or urinary fluoride differences between treatment groups were measured. In total 16 studies were included (10 RCTs, 5 CTs and 1 balance) only one of which (Villa *et al*, 2009) was assessed as being at a moderate, rather than a high risk of bias. There were just three studies which were conducted over the longer term (>48 hour samples for each treatment) and all but one of these studies was conducted in adult subjects.

Fluoride supplement studies generally found that the form of fluoride provided (type of preparation and/ or chemical form) did not significantly affect bioavailability, with the exception of sustained release preparations which were found to significantly delay fluoride absorption as expected. Jeandel *et al* (1992) aimed to determine the effect of age on fluoride supplement metabolism, finding an impairment of urinary excretion, resulting in the serum

area under the curve being 1.7 times higher in older participants (65-75 years). In adults, food consumed within 30 minutes of a fluoride supplement was found to delay fluoride absorption time, reduce peak fluoride levels, and overall, result in lower fluoride levels in the plasma by 8-47% (McIntyre *et al*, 2001; Pak *et al*, 1990; Shulman and Vallejo, 1990; Warneke and Setnikar, 1993). One balance study (Ekstrand *et al*, 1994) found no significant difference in fluoride supplement absorption given i) with a feeding, or ii) one hour before feeding. This study was conducted in a small infant sample (n=4). All studies addressing meal influences were assessed as being at high risk of bias.

Specific nutrient interactions were only addressed in detail for calcium and sodium chloride (salt). One study found calcium (400 mg calcium supplement) significantly reduced the absorption of fluoride supplements (23 mg fluoride as slow release sodium fluoride) by 27% (Pak *et al*, 1990). However four other studies addressing calcium influences on fluoride absorption did not find any significant differences (Maguire *et al*, 2005; Setnikar and Maurer, 1990; Shulman and Vallejo, 1990; Villa *et al*, 2009). With increasing ingestion of fluoridated salt, fluoride was retained by the body to a greater extent, or incompletely absorbed ($p<0.05$) (Nath *et al*, 1992). However, fluoride intake from fluoridated salt was reduced in comparison with fluoridated milk and water, due to salt loss in cooking (Toth *et al*, 2005).

Goyal *et al* (1998) considered fluoride bioavailability from typical meals eaten in different regions of India and found it to be relatively low (1.6–31.7%). Bioavailability was highest in the carbohydrate rich meal, but this diet contained the lowest amount of total fluoride (1.53 mg fluoride).

STATUS MARKERS (TABLE 6)

Establishing fluoride status by the measurement of appropriate status biomarkers is fundamental in studies of fluoride metabolism. Overall six non-randomised controlled trials and one cross-sectional study were included, all of which were assessed as being at high risk of bias. Studies of fluoride intake-status relationships largely measured 24 hour urinary excretion, although one study (Buzalaf *et al*, 2006) measured content of fluoride in nails. Buzalaf *et al* found the concentration of fluoride in nails, and lag time for appearance, differed significantly between fingers and toes, indicating that this should be taken into account when using nails as a measure of fluoride status. Studies assessing 24 hour urine as a status marker, typically found fluoride excretion to be approximately 30% of total intake. Villa *et al* (2008) found levels to be higher at 69%, however this study was conducted in adults (20-40 years), whilst others were in children (2-6 years) and young adults (21 ± 1 years). Some of the studies focusing on fluoride bioavailability and metabolism (**Table 5**) also measured fractional urinary excretion rate. The majority of these studies were conducted in young to middle aged adults in which approximate average fractional urinary excretion rates ranged from 40-70%. Villa *et al* (2008) found diurnal average urinary fluoride excretion (7 a.m.–6 p.m.) to be significantly lower than nocturnal excretion (6 p.m.-7 a.m. of the following day). Generally studies were in agreement that fluoride intake and excretion were well correlated, although

there was a wide variability between- and within- subjects. Franco *et al* (2005) was the only cross-sectional study included which measured usual dietary and dentifrice intake and related this to urinary excretion. Dentifrice contributed the largest proportion to total fluoride intake. One study (Watanabe *et al*, 1995) directly compared 24 hour urinary fluoride with spot urine samples, and confirmed that 24 hour collections were a more reliable indicator of fluoride intake.

POLYMORPHISMS (TABLE 7)

Whilst it is not the remit of this report to look at adverse effects relating to the consumption of doses above the UL, one case-control study was included on polymorphisms (prevalence >5%), which may potentially be of interest in the setting of DRVs for vulnerable individuals. The case-control study was published in two papers (Ba *et al*, 2009; Huang *et al*, 2008) assessing different polymorphisms of osteocalcin and collagen genes, but conducted in the same participant study group. Polymorphisms in osteocalcin HindIII and in collagen COL1A2 Rsa1 were not significantly associated with risk of dental fluorosis. For those carrying the homozygous PP for COL1A2 PvII, a significantly increased risk of dental fluorosis was found in comparison with genotype pp, but this relationship was only found within an endemic fluorosis area (fluoride levels in water >2 ppm). The trial was assessed as being at high risk of bias and was conducted in a relatively small sample of children (n=240). There was insufficient evidence to draw conclusions on the importance of polymorphisms in establishing dietary requirements.

BREAST MILK CONCENTRATION (TABLE 8)

All nine breast milk studies included were cross-sectional in design. The concentration of fluoride was highly variable ranging from 4.56-513 µg/l. Some articles suggested reasons for differences, such as living within an area supplied by fluoridated water. Difficulties in accurately determining levels of fluoride in breast milk and methodological variations were also noted (Parr *et al*, 1991; Sener *et al*, 2007). The most common procedure for fluoride analysis is using an ion-selective electrode to quantify free fluoride anion (Sener *et al*, 2007), however only Pasternak *et al* (1998) shows values for free, bound and total fluoride separately. Only one study (Opinya *et al*, 1991) directly recorded total maternal dietary intake, using 24 hour weighing of foods and beverages and analysis of samples for fluoride content. This study did not find a significant correlation between fluoride consumption and levels of fluoride in breast milk. All breast milk studies were assessed as being at high risk of bias, other than Opinya *et al* (1991) which was assessed as being at moderate risk of bias.

Conclusions

Articles from January 1990 to March 2011 have been systematically searched and reviewed using a standard protocol, tailored for the specific issues relevant to fluoride, with the aim of collating and assessing the body of evidence for fluoride relevant to setting DRVs.

A total of 43 studies met the inclusion criteria. Health endpoints included in this systematic review were tooth and bone health. Two studies relating to bone health were included (1 SR [that included 3 other SRs and 3 cross-sectional studies]; plus an additional nested case-control). There were eight studies assessing tooth health (5 SRs [including between them 6 other SRs; 18 RCTs; 1 cohort; 11 cross-sectional studies]; plus an additional 2 RCTs and 1 CT). Tooth health studies were mainly conducted in children. Only one study reported on the response of a health biomarker (in this case, leptin), to supplemental fluoride intake.

There were 16 studies which were focused on fluoride bioavailability and metabolism, all but one of these were conducted in adult subjects and only three were conducted over the longer-term (>48 hours). There were seven studies included which focused on measuring fluoride status marker changes following fluoride consumption, but the biomarkers were limited to urinary fluoride (6) and nail fluoride (1). The majority of articles (37) were assessed as being at high risk of bias, with the remainder at moderate (6) and low risk of bias (2).

There were relatively few studies of good quality regarding fluoride intake, status and/or health endpoints. For biokinetic data, there was a lack of well-conducted balance studies and long-term supplementation trials, particularly for children, elderly and pregnant women. Studies addressing bone and tooth health were of a higher quality (2 at low risk of bias and 4 at moderate risk of bias). Data were suggestive of a protective role for fluoride in the reduction of dental caries, but systematic reviews in this area acknowledged a lack of high quality intervention trials measuring total fluoride intake, and data were lacking for adult groups.

Overall, there was a lack of high quality evidence upon which DRVs may potentially be based for fluoride.

Table 2. Bone health

Reference	Study type	n	Age	Sex	Intervention and control or exposure	Dietary intake estimation	Follow up time	Results: effect, mean, SD, RR/OR/HR confidence interval, etc	Overall results	Risk of bias
Feskanich D *et al*, 1998	Nested case-control	492 (b)	30-55 years	492F	Nail clippings taken at baseline, participants followed up to determine if nail F is an indicator of bone health	FFQ	6 years	Women with F levels >5.50 ppm (versus <2 ppm) had odds ratios of 0.8 (95% CI 0.2,4.0) for hip fracture OR: 1.6 (95% CI=0.8-3.1) for forearm fracture	Results were equivocal and not dose-dependent	Low
NHMRC, 2007 (a)	SR	6 studies	Any	M & F	Hierachy of evidence for fluoridated milk, salt or water: cohort studies; case-control studies; comparative cross-sectional studies	Not reported	Not reported	No studies met the inclusion criteria for milk or salt. 3 SRs and 3 cross-sectional studies were included re: water. McDonagh (2000) SR forms the basis of evidence finding little evidence for positive or negative effects. Univariate analysis resulted in a pooled estimate of 1.00 (95% CI: 0.94-1.06), with significant heterogeneity between studies. Other studies support the findings of the SRs, although suggest optimal fluoridation levels of 1 ppm may result in lower risk of fracture	There was little evidence to support a beneficial effect of fluoridation in improving bone health. Recommended target fluoridation at level 0.6-1.1 ppm	Moderate

(a): NHMRC also included literature relating to tooth health as described in **Table 3**

(b): From Nurses' Health Study cohort of 121700, of which 62641 met inclusion criteria. Subsequently 246 participants were identified with fractures and were age matched with 246 controls at which point nail clippings were analysed for fluoride

Table 3. Tooth health

Reference	Study type	n	Age	Sex	Intervention and control or exposure	Dietary intake estimation	Follow up time	Results: effect, mean, SD, RR/OR/HR confidence interval, etc	Overall results	Risk of bias
Espelid I, 2009 (Included NHMRC, 2007 and Yeung *et al*, 2005 SRs and Lin and Tsai, 2000 RCT) (Included 3 RCTs which were also included within Ismail and Hasson, 2008 SR: Driscoll *et al*, 1979; Kallestal, 2005; Stephen *et al*, 1978)	SR	10 studies	22 months–12 years (a)	M & F	SRs or RCTs Fluoridated milk, salt, tablets, drops (no dose levels set)	Not reported	2-8 years (a)	Milk: 1 SR (Yeung, 2005) Insufficient evidence to draw conclusions. Salt: 2 SRs- (NHMRC, 2007 as tabulated and Swedish Council, 2002). Insufficient evidence to draw conclusions. Tablets/ drops: 7 RCTs included: 3 NS. 4 significant effects in risk reduction of DMFS/ DMFT (dmfs/ dmft) (F ≤2 mg/d). Difficulties in design of studies limits the strength of evidence	Insufficient studies with good quality evidence, although findings support caries risk reduction	High
Griffin S *et al*, 2007	SR	9 studies	Mean: 20+ years	M & F	Fluoridated water with a concurrent control and sufficient information to extrapolate findings to all 28 teeth (no dose levels set). (study also assesses dentifrice)	Not reported	≥1 year or if cross-sectional, subjects living in study areas for	9 water fluoridation studies were included (8 cross-sectional, 1 prospective cohort; n=7853 participants), with combined effectiveness significant (p<0.001). For the 7 studies including only lifelong residence (n=5409), RR was 0.654 (95% CI: 0.490-	Fluoridation at levels of 3.5-0.7 ppm v 0.1-0.7 ppm was found to be beneficial in caries prevention in adults	Moderate

Reference	Study type	n	Age	Sex	Intervention and control or exposure	Dietary intake estimation	Follow up time	Results: effect, mean, SD, RR/OR/HR confidence interval, etc	Overall results	Risk of bias
							majority of their lives	0.874), prevented fraction of 34.6% (95% CI: 12.6-51.0%). Heterogeneity was not an issue when the 5 studies published after 1979 (n=2530) were combined. The summary-prevented fraction was 27.2% (95% CI: 19.4-34.3%)		
Ismail A and Hasson H, 2008 (Included Leverett *et al*, 1997 RCT) (Included 3 RCTs which were also included within Espelid, 2009 SR: Driscoll *et al*, 1979; Kallestal, 2005; Stephen *et al*, 1978)	SR	12 studies	0-16 years	M & F	Randomised studies, longitudinal in design F tablets, lozenges or drops (no dose levels set)	Not reported	2-7.5 years (a)	8 studies reported significantly lower caries within the intervention group (F $\leq$1.5 mg/d). 4 studies did not report significant differences	Evidence was weak, but findings support the effectiveness of F from school age on (permanent teeth). However, evidence is less strong in the primary teeth	Moderate
Leverett D *et al*, 1997 (Included within	RCT	798 (at 5 year follow up)	Pre-natal – 5 years	M & F	Prenatal supplements of 2.2 mg NaF (1 mg F/d) or placebo	Not reported	5-6 years	dfs per 1000 surfaces yr 3: 2.7 for F group and 1.3 for placebo RR 2.07 (95% CI 0.82-5.24)	Findings do not support the hypothesis that prenatal F has a strong caries-preventive effect	Moderate

Reference	Study type	n	Age	Sex	Intervention and control or exposure	Dietary intake estimation	Follow up time	Results: effect, mean, SD, RR/OR/HR confidence interval, etc	Overall results	Risk of bias
Ismail and Hasson, 2008 SR). (Sa Roriz Fonteles *et al*, 2005 follows up this trial)					beginning with the 4th month of pregnancy. Post natal supplements were provided for all children (drops 0-2 years; 0.5 mg daily tablets 2-3 years) Parallel, double-blinded			yr 5: 5.2 for F group and 5.7 for placebo RR 0.90 (95% CI 0.41-1.97). Differences NS. 92% F children remained caries free and 91% placebo group		
Lin Y and Tsai C, 2000 (Included within Espelid, 2009 SR)	RCT	140	22-26 months	73M & 67F	1) No supplements (n=44) 2) Tablets (0.25 mg F/d) (n=46) 3) Drops (0.25 mg F/d) (n=50) Parallel, single-blinded	Not reported	2 years	<u>DMFT</u> Tablet and liquid groups developed 52.2% and 72.3% fewer new DMFT and showed lower caries increment (p=0.010 and p=0.001) respectively. When compared to the tablet group, the liquid group showed a 41.9% reduction (NS) <u>DMFS</u> Tablet and liquid groups developed 50.9% and 81.4% lower caries increment (p=0.065 and p=0.002). When compared to the tablet group, the liquid	F supplements significantly reduced caries risk. Drops were a slightly better vehicle (NS)	High

Reference	Study type	n	Age	Sex	Intervention and control or exposure	Dietary intake estimation	Follow up time	Results: effect, mean, SD, RR/OR/HR confidence interval, etc	Overall results	Risk of bias
								group showed a 62.2% reduction (NS)		
Mulyani D and McIntyre J, 2002	CT	176	7-19 years	99M & 77F	1) Fluoridated sugar 10 ppm NaF ~ 0.6 mg F/d (n=57) 2) Sugar (n=119) Parallel, double-blinded	Estimated from total consumed within the orphanage / boarding facilities	18 months	Urinary F doubled in test group ($p<0.05$). Change in DMFS score in controls was 1.47 (SD 1.69, SE 0.24) and 0.30 in test group (SD 0.63, SE 0.07), significant at $p<0.05$	Significant inhibition of caries development was found in the test group	High
NHMRC, 2007 (b) (Included Yeung *et al*, 2005 SR)	SR	6 studies	Any	M & F	Hierachy of evidence for fluoridated milk, salt or water: cohort studies; case-control studies; comparative cross-sectional studies For caries outcome, epidemiological evidence for fluoridated water: ≥2 levels of fluoridation at 2 points in time	Not reported	Not reported	Milk: 1 SR included (Yeung, 2005 as tabulated). And 2 cross-sectional studies. Insufficient evidence Salt: No studies met inclusion criteria Water: 2 key SRs and 1 original trial (comparative cross-sectional). McDonagh (2000) SR forms the basis of evidence: included 26 studies all moderate quality. Pooling resulted in 15.4% mean difference (95% CI 10.8, 20.1) of caries free individuals, and a mean positive difference in dmft/DMFT score of 2.3 (1.8, 2.8), $p<0.001$ in water fluoridated versus control	The existing body of evidence strongly suggested water fluoridation is beneficial in caries prevention. Recommended target fluoridation at level 0.6-1.1 ppm	Moderate

Reference	Study type	n	Age	Sex	Intervention and control or exposure	Dietary intake estimation	Follow up time	Results: effect, mean, SD, RR/OR/HR confidence interval, etc	Overall results	Risk of bias
								areas. After adjustment for confounders: 14.3% (95% CI 6.7, 21.9) and mean difference in dmft/ DMFT score of 2.61 (2.31, 2.91), p value not reported. Heterogeneity was high between studies (p<0.001). Other studies identified did not change these conclusions		
Sa Roriz Fonteles C *et al*, 2005 (Follow up of Leverett *et al*, 1997)	RCT	185	Pre-natal–5 years	M & F	Prenatal supplements of 2.2 mg NaF (1 mg F/d) (n=585) or placebo (n=590) beginning with the 4th month of pregnancy. Post natal supplements were provided for all children (drops 0-2 years; 0.5 mg daily tablets 2-3 years) Parallel, double-blinded	Not reported	Unclear	No significant differences between control and treatment groups: Mean F concentrations (µg/cm3) and SEM for Intervention group: Surface enamel: 3,790 (260) Body enamel: 1,331 (88) Dentine: 380 (28) Control group: Surface enamel: 3,430 (189) Body enamel: 1,350 (114) Dentine: 378 (29)	F exposure during the prenatal period offered no additional measurable F uptake by dental tissue other than that attributable to postnatal F alone	High
Yeung A *et al*, 2005	SR	2 studies n=353	3-6 years (a)	M & F	RCTs studies only Fluoridated milk or	Not reported	>3 years	Maslak, 2004 (2.5 mg F/l or ~ 0.5 mg F/d) Significant reduction in the	Insufficient studies with good quality evidence. However, the	Low

Reference	Study type	n	Age	Sex	Intervention and control or exposure	Dietary intake estimation	Follow up time	Results: effect, mean, SD, RR/OR/HR confidence interval, etc	Overall results	Risk of bias
(Included within Espelid, 2009 and NHMRC, 2007 SRs)					un-fluoridated milk			DMFT (78.4%, p<0.05) after 3 years, and in dmft 31.3%, p<0.05) Stephen, 1984 (7.5 mg F/l or ~1.5 mg F/d) Significant reduction in DMFT only in year 4 (35.5%, p<0.02) and year 5 (31.2%, p<0.05)	included studies suggested fluoridated milk was beneficial to school children	

(a): Data relates to study findings within broad inclusion criteria

(b): NHMRC also included literature relating to bone health as described in **Table 2**

Table 4. Biomarkers of disease

Reference	Study type	n	Age	Sex	Intervention and control or exposure	Dietary intake estimation	Follow up time	Results: effect, mean, SD, RR/OR/HR confidence interval, etc	Overall results	Risk of bias
Oral B and Ozbasar D, 2002	RCT	101	Mean 52.7 ± 2.2 years	101F	1) NaMFP (25 mg/d, or 3.29 mg F/d) with BMI <25 (n=29) 2) NaMFP (25 mg/d, or 3.29 mg F/d) with BMI ≥25 (n=26) 3) No NaMFP with BMI ≥25 (n=24) 4) No NaMFP with BMI <25 (n=22) Parallel. Blinding not reported	Not reported	12 months	Plasma leptin was slightly reduced in treated group (NS) Bone mineral density was increased in the spine of treated women significantly (2%)- no other details given including method of analysis	F treatment did not significantly change plasma leptin concentrations Leptin levels were significantly higher in obese, than in non-obese women ($p<0.001$)	High

Table 5. Bioavailability and metabolism

Reference	Study type	n	Age	Sex	Intervention and control or exposure	Dietary intake estimation	Follow up time	Results: effect, mean, SD, RR/OR/HR confidence interval, etc	Overall results	Risk of bias
Ekstrand J *et al*, 1994	Balance study	4	65-422 days	2M & 2F	3 regimens: no F supplement (A); supplement 0.25 mg NaF/d in glucose solution (0.11 mg F); B) given with a feeding; C) between feedings (3 hours after first feeding and 1 hour before second) Cross-over: 72 h study period, 11 day washout. Blinding not reported Feedings consisted of formula/ baby foods (fruit, vegetables, cereal, meats)	Food sample: weighed record of intake & F conc. of foods	72 hour samples	Regimen A: mean F (SD): Intake: 20.5 (4.2) µg/kg/d Absorption: 90.1 (3.2)% intake Urinary excretion: 15.5 (1.9) µg/kg/d Faecal excretion: 2.0 (0.7) µg//kg/d Retention: 12.5 (13.8)% intake Regimen B: Intake: 46.0 (5.2) µg/kg/d Absorption: 88.9 (10.5)% Urinary excretion: 19.2 (2.7) µg/kg/d Faecal excretion: 4.9 (4.5) µg/kg/d Retention: 47.1 (14.7)% of intake of intake, and as % dose: 68.1 (14.8) Regimen C: Intake: 48.9 (8.1) µg/kg/d Absorption: 96.0 (1.8)% Urinary excretion: 21.2 (3.2) µg/kg/d Faecal excretion: 2.0 (1.0) µg/kg/d Retention: 52.3 (6.7)%, and	Regimen B not significantly different from C. Significance of difference between A and other regimens not reported. Intake of F is the primary determinant of retention, regardless of whether F is provided by diet or derived predominantly from a single daily supplement	High

Reference	Study type	n	Age	Sex	Intervention and control or exposure	Dietary intake estimation	Follow up time	Results: effect, mean, SD, RR/OR/HR confidence interval, etc	Overall results	Risk of bias
								as % of dose: 73.0 (6.0). Intake and retention correlated (r=0.9507, p<0.001)		
Erlacher L *et al*, 1995	RCT	12	24-49 years	6M & 6F	76 mg MFP A: non-sustained release reference preparation B & C: sustained release formulations of MFP (after 2 hours 70% F release for B and 28% F release for C). All equivalent to 10 mg F and given with a standard breakfast Cross-over, 1 week washout period. Blinding not reported	Not reported	24 hour samples	<u>Plasma</u> C_{max} (ng/ml; mean ± SD) A 379.7 ± 77.5 B 165.6 ± 42.1 C 109.8 ± 48.2 T_{max} (h; median) A 0.5 B 1.2 C 4.0 AUC(ng/ml x h; mean ± SD) A 2373 ± 652.4 B 1487.2 ± 354.3 C 1369.1 ± 384.4 <u>Urinary excretion</u> Ue cumulative fluoride output (mg; mean ± SD) A 5.63 ± 0.73 B 3.58 ± 0.77 C 3.22 ± 1.12 Cl renal clearance (ml/min; mean ± SD) A 42.1 ± 10.7 B 43.0 ± 16.5	Sustained release preparations of MFP led to a significant decrease in F bioavailability and avoided high peak serum concentrations (p<0.001). Urinary excretion was significantly lower	High

Reference	Study type	n	Age	Sex	Intervention and control or exposure	Dietary intake estimation	Follow up time	Results: effect, mean, SD, RR/OR/HR confidence interval, etc	Overall results	Risk of bias
								C 40.5 ± 16.3		
Goyal A *et al*, 1998	CT	25	22-35 years	M & F	Regional representative meals in comparison to NaF (4.40 mg F): i) North Indian vegetarian- (4.00 mg F); ii) North Indian non-vegetarian- as for i) but with chicken (5.02 mg F); iii) South Indian- largely carbohydrate (1.53 mg F); iv) East Indian- containing fish (10.0 mg F); v) NaF 4.4 mg F (5 participants in each group) Parallel. Blinding not reported	Fixed diet	7 hour samples	AUC-p (µg F/100ml) (mean ± SD): i) 0.01 ± 0.05; ii) 0.23 ± 0.23; iii) 0.17 ± 0.41; iv) 0.19 ± 0.20; iv) NaF reference 4.24 ± 5.11. % Bioavailability relative to NaF: i) 1.6; ii) 14.4; iii) 31.7; iv) 7.5; v) 100 F (mg) absorbed in plasma: i) 0.06 (peaks at 3 hours); ii) 0.72 (peaks at 2 hours); iii) 0.48 (peaks at 1.3 and 4 hours); iv) 0.75 (peaks at 4 hours) ; v) immediate rise with peak at 45 minutes (16.36 µg F/100 ml)	Diets high in fish or chicken showed the highest F content. However, dietary analysis included bones which were discarded by participants. The bioavailability was highest for the carbohydrate based diet, although the F content for this diet was lowest. Overall authors conclude: the absorption of F from Indian diets was found to be low	High
Jeandel C *et al*, 1992	CT	27	65-75 years (n=15) 21-26	27M	Enteric coated tablet 50 mg NaF containing 22.6 mg F	Not reported	48 hour samples	Older participants (mean (SEM)) C_{max} (µmol/l): 596 (371) T_{max} (h): 3.12 (1.85)	Urinary flow rate and fractional F excretion rate were higher in older participants (NS).	High

Reference	Study type	n	Age	Sex	Intervention and control or exposure	Dietary intake estimation	Follow up time	Results: effect, mean, SD, RR/OR/HR confidence interval, etc	Overall results	Risk of bias
			years (n=12)		Parallel. Blinding not reported			AUC (µmol/h/l): 3410 (1667) $Cl \cdot f^{-1}$ (l/h): 8.91 (5.71) CL_{CR} (ml/min/1.73mm): 57.1 (20.5) CL_R (ml/mn): 43.7 (19.1) Fractional F excretion rate: 82.3% (46.4) <u>Younger participants</u> C_{max} (µmol/l): 420 (9.5) T_{max} (h): 2.84 (0.76) AUC (µmol/h/l): 2026 (322) $Cl \cdot f^{-1}$ (l/h): 11.4 (1.87) CL_{CR} (ml/min/1.73mm): 92.5 (20.1) CL_R (ml/mn): 64.9 (25.34) Fractional F excretion rate: 63.6% (25.2) <u>P value:</u> C_{max} (µmol/l): NS T_{max} (h): NS AUC (µmol/h/l): 0.0128 $Cl \cdot f^{-1}$ (l/h): 0.0128 CL_{CR}: 0.0018 CL_R (ml/mn): 0.018 Fractional F excretion rate: NS	Serum AUC of F was 1.7 times significantly higher (p=0.0128) and consequently $Cl \cdot f^{-1}$ was lower (p=0.0128). Impairment of Glomerular Filtration Rate in the elderly as measured by creatinine clearance (p=0.0018) suggests the dose of F should be adjusted by GFR	
Maguire A *et al*, 2005	RCT	20	Mean 25.7 ± 4.5 (20-35)	11M & 9F	500 ml water 1. Artificially fluoridated soft	Not reported	8 hour samples	C_{max} (ng/ml, mean 95% CI) 1) 15.3 (11.7, 19.0) 2) 14.8 (11.6, 18.1)	No significant differences between waters. However, large	High

Reference	Study type	n	Age	Sex	Intervention and control or exposure	Dietary intake estimation	Follow up time	Results: effect, mean, SD, RR/OR/HR confidence interval, etc	Overall results	Risk of bias
			years		water (1.01 mg F/l and 50 mg Calcium carbonate/l) 2. Artificially fluoridated hard water (0.97 mg F/l and 382 mg Ca/L) 3. Natural soft water (1.06 mg F/l and 63 mg Ca/L). 4. Natural hard water (0.91 mg F/l and 381 Ca/l) 5. NaF reference (1.02 mg F/l and 3 mg Ca/l) Double-blind cross-over, 1 week washout period			3) 12.5 (9.2, 15.7) 4) 14.2 (10.7, 17.8) 5) 14.2 (11.1, 17.3) T_{max} (mins, mean 95% CI) 1) 51.7 (46.9, 56.6) 2) 48.0 (43.7, 52.3) 3) 48.0 (43.7, 52.3) 4) 48.0 (43.1, 52.9) 5) 48.8 (44.3, 53.2) AUC (ng F/min/ml, mean 95% CI) 1) 1679 (1284, 2073) 2) 1566 (1175, 1958) 3) 1330 (1005, 1655) 4) 1440 (1071, 1810) 5) 1328 (991, 1664)	within- and between-subject variations in F absorption into the plasma	
McIntyre J *et al*, 2001	CT	3	Adults	1M & 2F	Fluoridated rice (mean fluoride 5.6 ppm, range 5.3-5.9 ppm), with 400 g consumed in male volunteer (2.24 mg F); 250 g in female volunteers (1.12 mg F)	Not reported	12 hour samples	Areas under the curve were relatively similar for NaF and fluoridated rice, although peaks in the plasma appeared more rapidly for NaF: peaks within 45 minutes compared to fluoridated rice: peaks within 3 hours	Further trials recommended. Authors acknowledge data is difficult to interpret due to the small sample size, differences in sampling and levels of consumption	High

Reference	Study type	n	Age	Sex	Intervention and control or exposure	Dietary intake estimation	Follow up time	Results: effect, mean, SD, RR/OR/HR confidence interval, etc	Overall results	Risk of bias
					On a separate occasion the male volunteer consumed 4.4 mg NaF in distilled water (2 mg F) Longitudinal. Blinding not reported					
Nath S *et al*, 1992	CT	12	Mean 27 ± 6 (18-37) years	5M & 7F	Salt with F at 250 ppm in 0.5 g packets as follows: Days 0-4: no fluoridated salt Days 5-6: 1 g salt (0.25 mg F) Days 7-8: 3 g salt (0.75 mg F) Days 9-10: 6 g salt (1.5 mg F) Days 11-12: 9 g salt (2.25 mg F) Longitudinal. Blinding not reported	Dietary history and recording foods eaten	12 days	Mean ± SE mg urinary F 0 g NaCL: 0.61 ± 0.036 1 g NaCL: 0.65 ± 0.055 3 g NaCl: 0.88 ± 0.058 ($p<0.05$ compared to 0, 1) 6 g NaCL: 1.21 ± 0.074 ($p<0.05$ compared to 0, 1, 3) 9 g NaCL: 1.52 ± 0.69 ($p<0.05$ compared to 0, 1, 3, 6). 40% of the ingested F dose is excreted from the additional amount supplied by NaCl	With increasing ingestion of fluoridated salt, F is retained by the body to a greater extent, or incompletely absorbed ($p<0.05$) Authors also note urinary excretion varied highly within individuals	High
Pak C *et al*, 1990	RCT	Study 1	25-62	F	Study 1:	Fixed diet	12 hour	<u>Study 1:</u>	Study 1	High

Reference	Study type	n	Age	Sex	Intervention and control or exposure	Dietary intake estimation	Follow up time	Results: effect, mean, SD, RR/OR/HR confidence interval, etc	Overall results	Risk of bias
		n=12 Study2 n=9	years		phase 1: 50 mg slow release NaF (23 mg F) without breakfast phase 2: 50 mg slow release NaF with 400 mg Ca without breakfast phase 3: 50 mg slow release NaF with breakfast phase 4: placebo without breakfast Study 2: phase 5: 50 mg slow release NaF with 400 mg Ca 2 hours beforehand phase 6: 50 mg slow release NaF with 400 mg Ca an hour after Cross-over. 4 day washout period. Blinding not-reported		samples	phase 1: (mean ± SD) C_{max} (ng/ml) 184 ± 33 T_{max} (h) 2.0 ± 1.0 AUC (ng/h/ml) 1314 ± 231 phase 2: C_{max} 135 ± 24 T_{max} 1.6 ± 0.7 AUC 961 ± 157 Phase 3 C_{max} 136 ± 33 T_{max} 3.6 ± 2.0 AUC 1096 ± 241 phase 4: results not given Phases 2 & 3 have lower AUC & C_{max} than phase 1 (<0.05); t_{max} higher in phase 3 than phase 1 (p<0.05) Study 2 No significant difference in C_{max}, T_{max} ,$T_{1/2}$ and AUC for either phase	NaF with calcium resulted in 27% significantly lower F levels in the serum and lower peak levels than when administered alone (p<0.05) (time differences to reach peak levels were NS) NaF with a meal, resulted in a 17% significantly lower F level in the plasma, lower peak levels and also a significantly longer time to reach peak F levels (p<0.05) Study 2 No significant differences between the two treatments	
Setnikar I and Maurer H, 1990	RCT	12	Mean 31.8 ± 5.4	4M & 8F	Solution of NaF in 80 ml water	Not reported	48 hour samples	NaF solution (mean ± SD) C_{max} 466 ± 71	The three products can be considered	High

Reference	Study type	n	Age	Sex	Intervention and control or exposure	Dietary intake estimation	Follow up time	Results: effect, mean, SD, RR/OR/HR confidence interval, etc	Overall results	Risk of bias
			(23-44) years		Solution of 100 mg sodium monofluorophosphate (Na_2FPO_3) in 80 ml water. Tablets with 100 mg sodium Na_2FPO_3 and 1250 mg calcium carbonate (all equivalent to 13.2 mg F) Cross-over. 7 day washout period. Blinding not reported			T_{max} 0.56 ± 0.20 AUC (0-48 h) 1862 ± 357 24 h urinary excretion (%): 51 ± 10 Na_2FPO_3 solution C_{max} 427 ± 155 T_{max} 0.73 ± 0.37 AUC (0-48h) 1771 ± 416 24 h urinary excretion (%): 46 ± 6 Na_2FPO_3 + Ca tablet C_{max} 412 ± 85 T_{max} 0.5 ± 0.10 AUC (0-48 h) 1711 ± 355 24 h urinary excretion (%): 47 ± 8 No significant difference between plasma concentration, AUC, C_{max}, $T_{max,}$ urinary excretion, after each of the three products	bioequivalent according to the Westlake criteria. Urinary excretion did not differ between the three products, thus confirming their bioequivalence	
Setnikar I *et al*, 1998	RCT	Study 1: n=18 Study 2: n=20	18-45 years	38M	Reference preparation 1(ref1):76 mg tablet sodium monofluorophosphate ($Na_2FPO_{3;}$ 10 mg elemental F) + 500 mg Ca	Not reported	Study 1 36 hour samples Study 2 48 hour samples	Study 1: Test1: C_b (basal F- ng/ml): 6 (5-7) AUC (ng/ml x h): 1054 (941-1167) C_{max} (ng/ml): 351 (324-377) T_{max} (h): 0.51 (0.49-0.54) Ae% (dose excreted- 36 h):	Test1 was found equivalent to Ref1 with regard to rate and extent of bioavailability of F in serum. Only T_{max} was significantly lower in test 1 ($p<0.05$)	High

Reference	Study type	n	Age	Sex	Intervention and control or exposure	Dietary intake estimation	Follow up time	Results: effect, mean, SD, RR/OR/HR confidence interval, etc	Overall results	Risk of bias
					Reference preparation 2 (ref2): effervescent tablet for oral solution 76 mg Na_2FPO_3, 2940 mg calcium lactogluconate & 300 mg calcium carbonate Test preparation 1 (test 1): chewable tablets, 76 mg Na_2FPO_3 and 1250 mg calcium carbonate. Test preparation 2 (test2): Effervescent tablets for oral solution containing 76 mg Na_2FPO_3 and 1250 mg calcium carbonate All equivalent to 10 mg F and 500 mg elemental Ca Study 1: ref1 v test1			44 (40-47) Ref1: C_b (ng/ml): 6 (5-8) AUC (ng/ml x h): 1204 (1037-1370) C_{max} (ng/ml): 336 (298-374) T_{max} (h): 0.78 (0.62-0.94) Ae%: 43 (39-47) Study 2: Test2 C_b (ng/ml): 1 (0-3) AUC (ng/ml x h): 1115 (1031-1198) C_{max} (ng/ml): 291 (266-316) T_{max} (h): 0.70 (0.55-0.85) Ae% (dose excreted- 48 h): 47 (41-51) Ref2 C_b (ng/ml): 4 (1-7) AUC (ng/ml x h):1067 (971-1162) C_{max} (ng/ml): 303 (280-326) T_{max} (h): 0.66 (0.62-0.71) Ae%: 51 (48-56)	Test2 was found bioequivalent in rate and extent to Ref2. All parameters NS All had short lag time, a rapid absorption, a C_{max} of F of 291-351ng/ml (NS between preparations) reached 30-75 min after administration, and a terminal t1/2 of 6-14 h/ About 50% of the absorbed F was eliminated with the urine 9from 0 to ∞ time). The renal clearance 65 ml/min	

Reference	Study type	n	Age	Sex	Intervention and control or exposure	Dietary intake estimation	Follow up time	Results: effect, mean, SD, RR/OR/HR confidence interval, etc	Overall results	Risk of bias
					Study2: ref2 v test2 Each study: 2 way cross-over. 6 day washout period. Non- blinded					
Shulman E and Vallejo M, 1990	RCT	10	18-35 years	10M	Session A: 2 x 1.1 mg NaF (1 mg f) Session B: 2 x 1.1 mg NaF 30 min after fixed lunch (15 min)- beef and turkey sandwiches, crisps, orange juice and an apple. Session C 2 x 1.1 mg NaF 15 min after 6 oz whole milk Session D Lunch with no fluoride treatment Cross-over, 1 week washout period. Blinding not-reported	Not reported	180 min samples	C_{max} (mmol/l, mean ± SD) F: 0.024 ± 0.008 F & milk: 0.020 ± 0.009 F & lunch: 0.008 ± 0.008 Lunch: 0.002 ± 0.005. T_{max} (min, mean ± SD) F: 99.8 ± 10.0 F & milk: 96.0 ± 10.5 Fluoride & lunch: 65.3 ± 56.8 Lunch: 12.0 ± 38.0. Area under curve (cm2; mean ± SD) F: 1.63 ± 0.79 F & milk: 1.42 ± 0.70 F & lunch: 0.86 ± 0.68 Lunch: 0.22 ± 0.53	F absorption was 13% (NS) when given with milk than F alone. The addition of lunch to the F supplement reduced absorption by 47% ($p<0.05$ compared to milk & F group, but not F group alone). The consumption of lunch significantly reduced peak levels and delayed timing of maximum F absorption ($p<0.01$). Milk did not significantly reduce peak levels or absorption time when taken 15 min prior F Consumption of F supplements are better absorbed without solid foods and as far away	High

Reference	Study type	n	Age	Sex	Intervention and control or exposure	Dietary intake estimation	Follow up time	Results: effect, mean, SD, RR/OR/HR confidence interval, etc	Overall results	Risk of bias
									from a meal as possible	
Toth Z *et al*, 2005	CT	20	Mean 25.6 ± 7.4 (19-45) years	9M & 11F	30 day test periods (2 week washouts): 1. Normal diet (unclear if 2 weeks or 30 days for control period) 2. 4 g salt daily (1 mg F) 3. 200 ml milk (1 mg F) 4. Tablet (1.1 mg F) Longitudinal. Blinding not reported	Not reported	30 day test periods	<u>Baseline</u> (urinary F mg/l) Control: 0.286 ± 0.083 Salt: 0.218 ± 0.066 Milk: 0.247 ± 0.079 Tablets: 0.228 ± 0.077 <u>Final day</u> Control: 0.232 ± 0.036 Salt: 0.451 ± 0.233 Milk: 0.671 ± 0.319 Tablets: 0.610 ± 0.213	Urinary F changes within salt, milk and tablet groups all significant at $p<0.001$ Changes in salt group lower which authors note likely to be due to loss in cooking rather than any change in metabolism using different F vehicles	High
Van Asten P *et al*, 1996	RCT	13	Mean 65 ± 3 (61-70) years	7F & 6M	Film coated tablet 76 mg (MFP, equivalent to 10 mg F); Enteric coated tablet of 25 mg sodium fluoride (NaF_{or}, equivalent to 11.3 mg F); isoosmotic aqueous injection solution (4 ml) of 22.1 mg sodium fluoride (NaF_{iv}, equivalent of 10 mg F)	Not reported	48 hour samples	Mean ± SD MFP $T_{1/2}$: 8.3 ± 3.3 C_{max}: 344 ± 113 T_{max}: 1.1 ± 0.5 Absolute bioavailability: 102.8% 48 h urinary excretion (mean ± SD): 45% ± 8.0 Renal clearance (ml/min, mean ± SD): 49 ± 15 NaF_{or} $T_{1/2}$: 8.7 ± 2.7	The MFP formulation showed smaller variation, higher absolute bioavailability, higher peak levels and quicker peak times than NaF_{or} ($p< 0.001$). The lower urinary excretion of NaF_{or} is due to incomplete absorption	High

Reference	Study type	n	Age	Sex	Intervention and control or exposure	Dietary intake estimation	Follow up time	Results: effect, mean, SD, RR/OR/HR confidence interval, etc	Overall results	Risk of bias
					Cross over. 1 week washout period. Blinding not-reported			C_{max}: 142 ± 96 T_{max}: 4.6 ± 3.3 Absolute bioavailability: 64.2% 48 h urinary excretion (mean ± SD): 26.7% ± 12.4 Renal clearance (ml/min, mean ± SD): 47 ± 15 NaF_{iv} $T_{1/2}$: 8.3 ± 2.1 C_{max}: n/a T_{max}: n/a 48 h urinary excretion (mean ± SD): 43.7% ± 9.9 Renal clearance (ml/min, mean ± SD): 50 ± 17		
Villa A *et al*, 2009	RCT	60	20-40 years	60F	Recorded consumption of: 1) NaF reference solution (0.804 mg F/l). 2) Naturally fluoridated water made up to 0.801 mg F/l with NaF, Calcium concentration was 151 mg/l	Duplicate-plate samples, individual calculation of F intake from beverages	24 hour samples	Fractional urinary F excretion (NS) between treatment groups: 1) 0.69 ± 0.10 2) 0.67 ± 0.16 Average 24 h fractional urinary F excretion was 0.69 95% CI. 0.65-0.73	Absorption of F is not affected by water hardness	Moderate

Reference	Study type	n	Age	Sex	Intervention and control or exposure	Dietary intake estimation	Follow up time	Results: effect, mean, SD, RR/OR/HR confidence interval, etc	Overall results	Risk of bias
					Parallel, double-blinded					
Warneke G and Setnikar I, 1993	RCT	8	Mean 28.5 ± 2.1 (26-32) years	8M	10 mg F as NaFMP & 300 mg Ca as calcium gluconate and calcium citrate. Given either fasting or just after a standard meal (cheese and ham rolls, boiled egg, coffee and milk) Two-treatment, two-period, cross-over. 7 day washout period. Non-blinded	Not reported	48 hour samples	Mean ± SD <u>Fasting</u> Lag (h): 0.06 ± 0.02 C_{max} (ng/ml): 369 ± 109 T_{max} (h): 0.56 ± 0.12 AUC (ng/mlxh): 1150 ± 358 48 h corrected urinary excretion: 45.5% <u>After meal</u> Lag (h): 0.18 ± 0.08 C_{max} (ng/ml): 122 ± 19 T_{max} (h): 2.44 ± 1.02 AUC (ng/mlxh): 1060 ± 414 48 h corrected urinary excretion: 43.7% Relative F absorbed from fasting conditions versus after a meal: 0.96 (90% CI: 0.88-1.03). Relative bioavailability 0.91: (90% CI: 0.74-1.13)	The meal did not significantly influence urinary excretion or the amount of absorbed F; however meals did significantly delay the rate of absorption and peak levels in the plasma ($p<0.01$ for lag time, $p<0.001$ for C_{max} and T_{max}.	High
Whitford G *et al*, 2008	RCT	10	24-32 years	M & F	500 ml water 1) naturally fluoridated (0.67 mg F/l and 5.45 mg	Not reported	6 hour samples	<u>0.67 F mg/l (mean ± SE)</u> C_{max} (µmol/l) natural: 0.58 ± 0.06 T_{max} (h) natural: 0.80 ± 0.08	No significant differences regarding type of water at either a low dose, or a high	High

Reference	Study type	n	Age	Sex	Intervention and control or exposure	Dietary intake estimation	Follow up time	Results: effect, mean, SD, RR/OR/HR confidence interval, etc	Overall results	Risk of bias
					F/l, with 20 mg/l Ca 2) deionised water with NaF (to match both concentrations above) Cross-over. Single-blinded			AUC (µmol/h/l) natural: 1.01 ± 0.22 C_{max} (µmol/l) NaF: 0.53 ± 0.08 T_{max} (h) NaF: 0.93 ± 0.07 AUC NaF: 1.07 ± 0.23 <u>5.45 F mg/l (mean ± SE)</u> C_{max} (µmol/l) natural: 6.17 ± 0.34 T_{max} (h) natural: 0.67 ± 0.00 AUC natural: 13.98 ± 0.58 C_{max} (µmol/l) NaF: 7.07 ± 0.35 T_{max} (h) NaF: 0.74 ± 0.07 AUC NaF: 13.94 ± 1.07	dose level	

Table 6. Status markers

Reference	Study type	n	Age	Sex	Intervention and control or exposure	Dietary intake estimation	Follow up time	Results: effect, mean, SD, RR/OR/HR confidence interval, etc	Overall results	Risk of bias
Buzalaf M *et al*, 2006	CT	10	20-35 years	10M	1.8 mg F/ d for 30 days (as NaF solution)- expected to double the background F intake Longitudinal. Blinding not reported	Duplicate diet	30 weeks	Increase in F in fingernails 84 days from baseline (NS); in toenails increases were significant ($p<0.05$) at 112 - 140 days from baseline. Peak F level at 20 weeks 1.9 µg/g Lag time: 123.05 ± 47.00 (95% CI: 103.60-143.50)	Growth rate and nail length (for each digit) must be taken into account when considering the use of nails as biomarkers. Big toenails are the most suitable nails for F detection due to their large size, relatively fast growth rate and being less prone to environmental contamination	High
Franco A *et al*, 2005	Cross-sectional	120	48-59 months	M & F	Usual F dietary intake	Duplicate diet	3 days	Global mean F intake (mg/kg/d) 0.004 (4.1%) beverages; 0.026 (27.1%); foods; 0.068 (68.8%); toothpaste. Total mean intake of 0.098 ± 0.075 (mg/kg/d), or 1.58 ± 1.14 mg F/d. One city (Cartagena) had an intake significantly lower than the other three cities 0.053 ± 0.018 mg F/kg/d ($p<0.01$). Mean (SD) F excretion (24 hours) was 0.414 mg/d	Where F intake was lower (1 city out of the 4 areas), this was reflected by a lower excretion level Dentifrice contributes the greatest proportion of F intake	High

Reference	Study type	n	Age	Sex	Intervention and control or exposure	Dietary intake estimation	Follow up time	Results: effect, mean, SD, RR/OR/HR confidence interval, etc	Overall results	Risk of bias
								(0.266), 95% CI= ± 0.053. Cartagena had an excretion rate which was significantly lower ($p<0.01$) than the other cities at 0.290 (0.177). Mean fractional urinary F excretion (output over intake) 0.33 (0.21), 95% CI= ±0.04, which was not statistically different in any of the 4 areas		
Ketley C and Lennon M, 2000	CT	8	4-5 years	M & F	0.5 mg F tablet daily for 2 days, following F free diet ie abstaining from tea, fluoridated milk and fish Longitudinal. Blinding not reported	Dietary record	4 days	Assuming complete absorption of 0.5 mg tablet, 24 h fractional F excretion was calculated as response from standard dose minus the background F excretion expressed as a % of 0.5 mg. The range was 22.6-38.9%, mean 30.1% ± 5.49, 95% CI 26.1,34.2	F levels in the urine peak following consumption of fluoridated dentifrice or foods relatively high in F e.g. school milk supplemented with fluoride. There was considerable inter-subject variation	High
Ketley C and Lennon M, 2001	CT	13	5-6 years	M & F	Usual dietary intake for 1 week. For the test period subjects were asked to consume a F free diet, with the exception of F	Dietary record	12 days	Linear regression indicates F excretion (24 h samples) rising in response to increasing F dose. Equation for the line was Fex=0.2220 Fin + 0.134 and the 95% CI interval for the slope was	Fractional urinary F excretion calculated at 39% for usual diet using the regression line. However, data was scattered due to varying individual	High

Reference	Study type	n	Age	Sex	Intervention and control or exposure	Dietary intake estimation	Follow up time	Results: effect, mean, SD, RR/OR/HR confidence interval, etc	Overall results	Risk of bias
					supplements provided: Study group 1 (n=9): day 1, 0.5 mg F; day 2, 1 mg F; day3, 1.5 mg F; day 4, 2 mg F. Study group 2 (n=4): day 1, 0.5 mg F; day 2, 0.75 mg F; day 3, 1.25 mg F; day 4, 1.5 mg F. The doses were split into 2-3 smaller doses to simulate varying daily conditions Longitudinal. Blinding not reported			0.163,0.278. (Although fractional F urinary excretion figure is higher for lower F doses)	excretion patterns	
Villa A *et al*, 1999	CT	48	3-5 years	23M & 25F	Identical control and test day lunch and dinners. 1 mg F (in 50 ml orange juice) added for test day Longitudinal, blinding not	Fixed diet	2 days	Excretion of F (24 h samples) ingested from the single F dose presented an average value of 30.7% (95% CI: 28.9-32.5) The average rate of F excretion in the first 7 h was significantly greater on the	Further studies to assess F fractional excretion under conditions of usual dietary intake are needed to establish the use of urine as a biomarker	High

Reference	Study type	n	Age	Sex	Intervention and control or exposure	Dietary intake estimation	Follow up time	Results: effect, mean, SD, RR/OR/HR confidence interval, etc	Overall results	Risk of bias
					reported			test day than the control day ($p<0.0001$), whilst there was no significant difference for the consecutive 17 h period between groups		
Villa A *et al*, 2008	CT	60	20-40 years	60F	Standardised breakfast, lunch and dinner to simulate usual dietary F intake. Non fluoridated dentifrice was provided Diurnal period (~11 hours from 07.00-18.00): mean F intake of 1.06 mg ± 0.36 (95% CI: 0.97-1.15) Nocturnal period (~13 hours from 18.00-07.00): mean F intake of 0.76 ± 0.35 (95% CI: 0.67-0.95) Longitudinal. Blinding not reported	Duplicate-plate samples, individual calculation of F intake from beverages	24 hour samples	The average fractional urinary F excretion 24 h: 0.69 ± 0.15 (95% CI: 0.65-0.72). Diurnal period (~11 h from 07.00- 18.00): 0.46 ± 0.14 (95% CI: 0.42-0.50) Nocturnal period (~13 h from 18.00 to 07.00): 1.09 ± 0.48 (95% CI: 0.97-1.22)- significantly higher ($p<0.001$)	The diurnal average fractional urinary F excretion was significantly lower than the nocturnal one. The daily F retention was estimated as 20% of ingested F	High
Watanabe M *et al*, 1995	CT	8	Mean 21 ± 1	8F	Four different typical Japanese	Fixed diet	16 days	No marked change in urine in 24 h with an intake of	F in diet and 24 h urinary excretion were	High

Reference	Study type	n	Age	Sex	Intervention and control or exposure	Dietary intake estimation	Follow up time	Results: effect, mean, SD, RR/OR/HR confidence interval, etc	Overall results	Risk of bias
			years		diets. Diet days 1-3 contained approximately 1 mg F (day1: 0.79-1.00; day 2: 1.01-1.15; day 3: 0.86-1.04), whilst diet day 4 contained higher amounts (2.61-2.74 mg) Longitudinal. Blinding not reported			1.01 mg F. For 2.73 mg F urinary excretion was approx 0.2 ppm before dinner intake but reached 1.17 ppm at 3.5 hrs after dinner intake and this level continued to the next morning, and a relatively high level of 0.66 ppm was found at 8.30. Rates of urinary excretion overall ranged from 18.1%-35.3%	well correlated (r=0.95). 24 h urine samples did not correspond to spot samples, except where collected after meal intake. A correlation between spot urine and intake was only found when collected after meal intakes (r=0.75 or over) Good correlation was obtained between measured concentrations and concentrations corrected for specific gravity or creatinine	

Table 7. Polymorphisms

Reference	Study type	n	Age	Sex	Intervention and control or exposure	Dietary intake estimation	Follow up time	Results: effect, mean, SD, RR/OR/HR confidence interval, etc	Overall results	Risk of bias
Ba Y *et al*, 2009 (a)	Case-control	240	8-12 years	126M & 114F	75 cases and 69 controls in endemic fluorosis area (EFA): F level in drinking water >2 mg/l . 96 controls in Non-EFA, water <1 mg/l F	Not reported	N/A	Osteocalcin HindIII polymorphism was not significantly related to dental fluorosis risk	More studies required since multiple genes influence dental malformations	High
Huang H *et al*, 2008 (a)	Case-control	240	8-12 years	126M & 114F	75 cases and 69 controls in endemic fluorosis area (EFA): level in drinking water >2 mg/l F. 96 controls in Non-EFA, water <1 mg/l F	Not reported	N/A	Collagen COL1A2 Rsa1 polymorphism was not significantly related to dental fluorosis risk COL1A2 PvII: genotype PP had a significantly increased risk of dental fluorosis (OR = 4.85, 95% CI: 1.22-19.32), compared to those with pp in an endemic fluorosis area. However, the risk was not elevated when the control population was recruited from a non-endemic area (OR= 1.07, 95% CI: 0.45-2.52)	Larger studies and different population studies would be needed to confirm an association	High

(a): Different polymorphisms explored in the same participant group

Table 8. Breast milk concentration

Reference	No. Samples	Country	Total maternal intake (mg/day) Mean (range)	Stage of lactation	F level (µg/l) mean ± SD	median ± SD	range	Risk of bias
Chuckpaiwong S *et al*, 2000	65	Thailand	Not reported	Not reported	17 ± 20			High
Hossny E *et al*, 2003	60	Egypt	Not reported	Not reported	4.56 ± 2.47	3.23	1.9-11.4	High
Koparal E *et al*, 2000	57	Turkey	Not reported	4/5 days	19 ± 4.0		5-25	High
Opinya G *et al*, 1991	27	Kenya	22.1 (9.5-37.2)	10.2 months (0.5-44)	33		11-73	Moderate
Parr R *et al*, 1991 (WHO/IAEA project)	84 71 18 61 31 68	Guatemala Hungary Nigeria Philippines Sweden Zaire	Not reported	~3 months		9.4 ± 0.5 13.8 ± 0.8 24.7 ± 9.7 118 ± 13 17 ± 1.9 6.8 ± 0.6		High
Pasternak K *et al*, 1998 (a)	10	Poland	Not reported	Not reported	513 ± 55			High
Rahul P *et al*, 2003	20	India	Not reported	Not reported	80 ± 132		50-100	High
Sener Y *et al*, 2007	125	Turkey	Not reported	5-7 days	6 ± 2		3-11	High
Viswanathan G *et al*, 2010 (b)	15	India	Diet / Water N 4.5 (3.4-5.7) / 3.0 (2.3-3.8) F1 10.8 (8.2-13.4) / 7.9 (6.1-9.7) F2 19.3 (14.7-23.9) / 14.5 (11.1-17.8)	<1 month	40 ± 10 (N) 40 ± 10 (F1) 50 ± 10 (F2)			High

(a): Total fluoride. Free fluoride was 492 ± 56 µg/l and bound fluoride 21 ± 3µg/l

(b): Intakes only estimated for the whole population by household survey. N= normal fluoride area (water within 1 mg/l); F1= medium fluoride area (1-2 mg/l); F2= high fluoride area (>2 mg/l)

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Appendices

Appendix A: search strategies

Magnesium i) Medline (OVID SP), ii) EMBASE (OVID SP), iii) Cochrane CENTRAL

Potassium i) Medline (OVID SP), ii) EMBASE (OVID SP), iii) Cochrane CENTRAL

Fluoride i) Medline (OVID SP), ii) EMBASE (OVID SP), iii) Cochrane CENTRAL

Appendix B: glossary and abbreviations

APPENDIX A

SEARCH STRATEGIES: MAGNESIUM

i) Magnesium: Medline (OVID SP), 1990 to 3rd October 2011

#	Searches	Results
1	magnesium/ or magnesium chloride/ or magnesium hydroxide/ or magnesium sulfate/ or magnesium oxide/	65442
2	*nutritional support/ or *dietary supplements/ or nutritional requirements/ or nutritional status/ or *deficiency diseases/ or *diet/ or exp nutrition assessment/ or biological availability/ or intestinal absorption/ or milk, human/	172411
3	biological markers/	122319
4	2 or 3	292403
5	1 and 4	2035
6	magnesium deficiency/	3696
7	5 or 6	5479
8	((magnesium* or 24Mg or Mg24 or 25Mg or Mg25 or 26Mg or Mg26 or 28Mg or Mg28) adj3 (intake* or diet*)).ti,ab.	1389
9	((magnesium* or 24Mg or Mg24 or 25Mg or Mg25 or 26Mg or Mg26 or 28Mg or Mg28) adj3 (status or balance or blood or serum or plasma or erythrocyte* or muscle or bone or biomarker* or bio-marker* or urin* or faeces or faecal or feces or fecal)).ti,ab.	5262
10	((magnesium* or 24Mg or Mg24 or 25Mg or Mg25 or 26Mg or Mg26 or 28Mg or Mg28) adj3 (absorption* or bioavailab* or bioacces* or balance or deplet* or supplement*)).ti,ab.	1867
11	8 and 9	397
12	10 or 11	2109

13	7 or 12	6655
14	humans/	12102898
15	animals/	4883931
16	14 and 15	1293157
17	15 not 16	3590774
18	13 not 17	4516
19	limit 18 to (english language and yr="1990 -Current")	2135
20	limit 19 to (addresses or autobiography or bibliography or biography or dictionary or directory or festschrift or interactive tutorial or interview or lectures or legal cases or letter or news or newspaper article or portraits or video-audio media or webcasts)	129
21	19 not 20	2006
22	("cross section*" or cross-section*).mp. [mp=protocol supplementary concept, rare disease supplementary concept, title, original title, abstract, name of substance word, subject heading word, unique identifier]	190547
23	cross-sectional studies/	131582
24	22 or 23	190547
25	21 not 24	1909

ii) Magnesium: EMBASE (OVID SP), 1990 to 3rd October 2011

#	Searches	Results
1	*magnesium oxide/ or *magnesium acetate/ or *magnesium citrate/ or *magnesium chloride/ or *magnesium sulfate/ or *magnesium carbonate/ or *magnesium hydroxide/ or *magnesium salt/	9322
2	magnesium derivative/	1878
3	1 or 2	11146
4	*magnesium/	25022
5	3 or 4	35333
6	diet supplementation/ or dietary intake/ or exp nutritional requirement/ or nutritional status/ or nutritional deficiency/ or biological marker/	205727
7	5 and 6	1042
8	(magnesium* adj3 (supplement* or deplet* or balance)).ti,ab.	1764
9	magnesium*.ti,ab.	46403
10	(intake* or diet*).ti,ab.	489370
11	(status or serum or plasma or urin* or biomarker* or bio-marker*).ti,ab.	2219557
12	9 and 10 and 11	2854
13	8 or 12	4277
14	7 or 13	4861
15	animal/	1641803
16	human/	12498023
17	nonhuman/ or exp in vitro study/	5634595
18	15 or 17	7097673

19	16 and 18	2267711
20	18 not 19	4829962
21	14 not 20	3396
22	limit 21 to (english language and yr="1990 -Current")	2124
23	limit 22 to (book or book series or editorial or letter or note or trade journal or conference abstract or "conference review")	192
24	22 not 23	1932
25	("cross section*" or cross-section*).mp. [mp=title, abstract, subject headings, heading word, drug trade name, original title, device manufacturer, drug manufacturer, device trade name, keyword]	174640
26	cross-sectional studies/	55187
27	25 or 26	174640
28	24 not 27	1800

iii) Magnesium: Cochrane CENTRAL, 1990 to 3rd October 2011

ID	Search	Hits
1	MeSH descriptor Magnesium, this term only	970
2	MeSH descriptor Magnesium Compounds, this term only	34
3	MeSH descriptor Magnesium Chloride, this term only	42
4	MeSH descriptor Magnesium Hydroxide, this term only	205
5	MeSH descriptor Magnesium Oxide, this term only	57
6	MeSH descriptor Magnesium Sulfate, this term only	499
7	(#1 OR #2 OR #3 OR #4 OR #5 OR #6)	1627
8	(magnesium* NEAR/3 (intake* or diet* or fortif* or supplement* or deplet* or balance or status or serum or plasma or blood or erythrocyte or muscle or bone or urin* or fecal or faecal or feces or faeces or biomarker* or bio-marker*)):ti,ab	748
9	MeSH descriptor Dietary Supplements, this term only	4146
10	MeSH descriptor Nutritional Requirements explode all trees	442
11	MeSH descriptor Nutritional Status explode all trees	1256
12	MeSH descriptor Deficiency Diseases, this term only	73
13	MeSH descriptor Nutrition Disorders, this term only	356
14	MeSH descriptor Diet, this term only	3271
15	MeSH descriptor Nutrition Assessment explode all trees	514
16	MeSH descriptor Biological Markers, this term only	5796
17	(#9 OR #10 OR #11 OR #12 OR #13 OR #14 OR #15 OR #16)	14268
18	(#7 AND #17)	123

19	MeSH descriptor Magnesium Deficiency explode all trees	68
20	(#8 OR #18 OR #19)	805
21	(#20), from 1990 to 2011	634

SEARCH STRATEGIES: POTASSIUM

i) Potassium: Medline (OVID SP), 1990 to 28th September 2011

#	Searches	Results
1	Potassium/	91349
2	Potassium Deficiency/	1507
3	potassium chloride/ or potassium, dietary/ or potassium iodide/	18653
4	((potassium or 42K or K42 or 40K or K40) adj3 (intake* or diet* or supplement* or deplet* or status or bone or fecal or faecal or faeces or feces or muscle or biomarker* or bio-marker*)).ti,ab.	4371
5	dietary supplements/ or exp nutritional requirements/ or nutritional status/ or deficiency diseases/ or *diet/ or exp nutrition assessment/	109218
6	biological markers/ or Milk, human/	136329
7	1 or 2 or 3	109278
8	5 or 6	242271
9	7 and 8	1500
10	9 or 4	5534
11	(animals not (humans and animals)).sh.	3590774
12	10 not 11	3820
13	limit 12 to (english language and yr="1990 -Current")	1855

14	limit 13 to (addresses or autobiography or bibliography or biography or comment or dictionary or directory or editorial or festschrift or interactive tutorial or interview or lectures or legal cases or letter or news or newspaper article or portraits or video-audio media or webcasts)	69
15	13 not 14	1786
16	("cross section*" or cross-section*).mp. [mp=protocol supplementary concept, rare disease supplementary concept, title, original title, abstract, name of substance word, subject heading word, unique identifier]	190547
17	Cross-Sectional Studies/	131582
18	16 or 17	190547
19	15 not 18	1662

ii) Potassium: EMBASE (OVID SP), 1990 to 28th September 2011

#	Searches	Results
1	*potassium/	37775
2	diet supplementation/ or exp nutritional requirement/ or nutritional status/ or nutritional deficiency/ or diet/ or nutritional assessment/ or dietary intake/	242417
3	biological marker/	77924
4	2 or 3	317849
5	1 and 4	1034
6	(potassium* adj3 (intake* or diet or dietary or diets or supplement* or deplet* or status or urin* or muscle or biomarker* or bio-marker*)).ti,ab.	7056
7	5 or 6	7655
8	human/	12498023
9	animal/	1641803
10	nonhuman/ or exp in vitro study/	5634595
11	9 or 10	7097673
12	8 and 11	2267711
13	11 not 12	4829962
14	7 not 13	5483
15	limit 14 to (english language and yr="1990 -Current")	2371
16	limit 15 to (book or book series or conference abstract or "conference review" or editorial or letter or note or trade journal)	245

17	15 not 16	2126
18	("cross section*" or cross-section*).mp.	174640
19	cross-sectional studies/	55187
20	18 or 19	174640
21	17 not 20	2021

iii) Potassium: Cochrane CENTRAL, 1990 to 30th September 2011

ID	Search	Hits
1	MeSH descriptor Potassium explode all trees	1946
2	MeSH descriptor Potassium Chloride, this term only	271
3	MeSH descriptor Potassium Citrate, this term only	24
4	(#1 OR #2 OR #3)	2175
5	(Potassium NEAR/3 (intake* or diet* or fortif* or supplement* or deplet* or balance or status or urin* or muscle or biomarker* or bio-marker*)):ti,ab	666
6	MeSH descriptor Dietary Supplements, this term only	4146
7	MeSH descriptor Nutritional Requirements explode all trees	442
8	MeSH descriptor Nutritional Status explode all trees	1256
9	MeSH descriptor Deficiency Diseases, this term only	73
10	MeSH descriptor Diet, this term only	3271
11	MeSH descriptor Nutrition Assessment explode all trees	514
12	MeSH descriptor Biological Markers, this term only	5796
13	(#6 OR #7 OR #8 OR #9 OR #10 OR #11 OR #12)	14043
14	MeSH descriptor Potassium Deficiency explode all trees	17
15	(#4 AND #13)	123
16	(#15 OR #5 OR #14)	737
17	(#16), from 1990 to 2011	428

SEARCH STRATEGIES: FLUORIDE

i) Fluoride: Medline (OVID SP), 1990 to 21st March 2011

#	Searches	Results
1	fluorides/ or fluorine compounds/ or calcium fluoride/ or sodium fluoride/	24012
2	(fluorid* adj3 (intake* or diet* or fortif* or supplement* or milk* or deplet* or balance or status or hair or urin* or serum or plasma or bone or saliva or nail or biomarker* or bio-marker* or tablet* or capsule* or drop or drops or pill*)).ti,ab.	3096
3	dietary supplements/ or exp nutritional requirements/ or exp nutritional status/ or malnutrition/ or deficiency diseases/ or nutrition disorders/ or child nutrition disorders/ or infant nutrition disorders/ or *milk/ or diet/ or exp nutrition assessment/ or capsules/ or pharmaceutical solutions/ or exp tablets/	215067
4	biological markers/	110431
5	3 or 4	322794
6	1 and 5	1283
7	2 or 6	3703
8	Humans/	11581056
9	Animals/	4684192
10	8 and 9	1216951
11	9 not 10	3467241
12	7 not 11	3007
13	limit 12 to (English language and yr=”1990 –Current”)	1382
14	limit 13 to (addresses or autobiography or bibliography or biography or dictionary or festschrift or in vitro or lectures or legal cases or letter or news or newspaper article or portraits or video-audio media or webcasts)	41

15	13 not 14	1341

ii) Fluoride: EMBASE (OVID SP), 1990 to 21st March 2011

#	Searches	Results
1	*FLUORIDE/	13099
2	(fluorid* adj3 (intake* or diet* or fortif* or supplement* or deplet* or balance or status or hair or urin* or serum or plasma or bone or saliva or nail or biomarker* or bio-marker*)).ti,ab.	3015
3	supplementation/ or diet supplementation/ or dietary intake/ or exp nutritional requirement/ or nutritional status/ or nutritional deficiency/ or biological marker/	202677
4	1 and 3	330
5	2 or 4	3161
6	Humans/	12219843
7	Animals/	1649221
8	6 and 7	397859
9	7 not 8	1251362
10	5 not 9	2938
11	limit 10 to (English language and yr=”1990 –Current”)	1656
12	limit 11 to (book or book series or editorial or letter or note or trade journal)	57
13	11 not 12	1599

iii) Fluoride: Cochrane CENTRAL, 1990 to 21st March 2011

ID	Search	Hits
1	MeSH descriptor Fluorides, this term only	807
2	MeSH descriptor Fluorine Compounds, this term only	0
3	MeSH descriptor Calcium Fluoride, this term only	15
4	MeSH descriptor Sodium Fluoride, this term only	572
5	(#1 OR #2 OR #3 OR #4)	1201
6	(fluorid* NEAR/3 (intake* or diet* or fortif* or supplement* or milk* or deplet* or balance or status or hair or urin* or serum or plasma or bone or saliva or nail or biomarker* or bio-marker* or tablet* or capsule* or drop or drops or pill*)):ti,ab,kw	414
7	MeSH descriptor Dietary Supplements, this term only	3910
8	MeSH descriptor Nutritional Requirements explode all trees	434
9	MeSH descriptor Nutritional Status explode all trees	1225
10	MeSH descriptor Malnutrition, this term only	150
11	MeSH descriptor Deficiency Diseases, this term only	69
12	MeSH descriptor Nutrition Disorders, this term only	369
13	MeSH descriptor Child Nutrition Disorders, this term only	106
14	MeSH descriptor Infant Nutrition Disorders, this term only	61
15	MeSH descriptor Milk, this term only	755
16	MeSH descriptor Diet, this term only	3157
17	MeSH descriptor Nutrition Assessment explode all trees	502
18	MeSH descriptor Capsules, this term only	1166

19	MeSH descriptor Pharmaceutical Solutions, this term only	94
20	MeSH descriptor Tablets explode all trees	2412
21	MeSH descriptor Biological Markers, this term only	5475
22	(#7 OR #8 OR #9 OR #10 OR #11 OR #12 OR #13 OR #14 OR #15 OR #16 OR #17 OR #18 OR #19 OR #20 OR #21)	17731
23	(#5 AND #22)	70
24	(#6 OR #23)	434
25	(#24), from 1990 to 2011	302

APPENDIX B

Glossary / Abbreviations

ßCTX	C-terminal cross linking telopeptide of type I collagen
µg	Micrograms
µmol	Micromoles
AAS	Atomic absorption spectrometry
ANP	Atrial natriuretic peptide
AUC	Area under curve
B	Boron
BALP	Bone specific alkaline phosphatase
BMD	Bone mineral density
BMI	Body mass index
BP	Blood pressure
Ca	Calcium
Cal	Calories
CI	Confidence interval
cm	Centimetres
C_{max}	Maximum concentration
Cr	Chromium
Cr	Creatinine
CT	Non-randomised controlled trial
Cu	Copper
d	Day(s)
DASH	Dietary approaches to stop hypertension

DBP	Diastolic blood pressure
Dl	Decilitre
DMFT/DMFS	Decayed, missing and filled teeth/ surfaces (secondary teeth)
DRV	Dietary Reference Values
EC	European Commission
EC_{50}	ADP concentration causing 50% of the maximal initial rate
ECG	Electrocardiogram
EFSA	European Food Safety Authority
EI	Estimated intake
EURRECA	EURopean Micronutrient RECommendations Aligned
F	Fluoride
fDPD	Free deoxypyridinoline
FF	Filtration factor
FMD	Flow mediated dilation
fPYD	Free pyridinoline
g	Grams
GFR	Glomerular filtration rate
h	Hour
Hb	Haemoglobin
HDL	High density lipoprotein cholesterol
HOMA-IR	Homeostasis model assessment-estimated insulin resistance
HR	Hazard ratio
ICAP	Inductively coupled argon plasma spectrometry

ICP-MS	Inductively coupled plasma mass spectrometry
IOM	Institute of Medicine
IQR	Interquartile range
ISH	International Society of Hypertension
IV	Intravenous
IVGTT	Intravenous glucose tolerance test
K	Potassium
^{42}K	Potassium 42 isotope
Kcal	Kilocalorie
kg	Kilogram
l	Litre
LDL	Low density lipoprotein cholesterol
MAP	Mean atrial pressure
MCV	Mean corpuscular volume
mEq	Molar equivalent
MFP/NaMFP/Na_2FPO_3	Sodium monofluorophosphate (also known as e.g. disodiummonofluorophosphate)
mg	Milligrams
Mg	Magnesium
^{25}Mg	Magnesium 25 isotope
^{26}Mg	Magnesium 26 isotope
min	Minute
MJ	Megajoule
ml	Millilitre

mm Hg	Millilitres of mercury (measure of pressure)
mmol	Millimoles
mol	Moles
mOsm	Milliosmole
n	Number
Na	Sodium
NaCl	Sodium chloride
NaF	Sodium fluoride
NE	Norepinephrine
ng	Nanograms
NHMRC	National Health and Medical Research Council
nMBCE	Nanomoles bone collagen equivalent
Nmol	Nanomoles
N/R	Not reported
NS	Not significant
NTx	N-telopeptides of type I collagen
OC	Osteocalcin
OR	Odds ratio
p	Level of significance
P	Phosphorus
PINP	Procollagen type I N-terminal propeptide
ppm	Parts per million
PRISMA	Preferred reporting items for systematic review

PTH	Parathyroid hormone
PWV	Pulse wave velocity
QRS	A complex made up of 3 waves (Q, R and S-waves) which depict heart beats on an electrocardiogram
RBC	Red blood cells (erythrocytes)
RCT	Randomised controlled trial
RDA	Recommended Daily Allowance
RMR	Resting metabolic rate
RR	Relative risk
RVR	Renal vascular resistance
SBP	Systolic blood pressure
SCF	Scientific Committee for Food
SD	Standard deviation
SE	Standard error of the mean
SOD	Superoxide dismutase
SR	Systematic review
SRE	Salt-resistant
SS	Salt-sensitive
T_{max}	Time to maximum concentration
TOHP	Trials of hypertension prevention
UL	Upper limit of intake
WASO	Wake after sleep onset
WHO	World Health Organisation
WI	Water immersion to the neck

Zn	Zinc