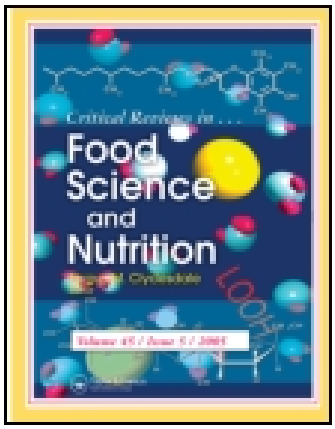

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EURRECA—Estimating Selenium Requirements for Deriving Dietary Reference Values

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EURRECA—Estimating Selenium Requirements for Deriving Dietary Reference Values

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Current reference values for selenium, an essential micronutrient, are based on the intake of selenium that is required to achieve maximal glutathione peroxidase activity in plasma or erythrocytes. In order to assess the evidence of relevance to setting dietary reference values for selenium, the EURRECA Network of Excellence focused on systematic searches, review, and evaluation of (i) selenium status biomarkers and evidence for relationships between intake and status biomarkers, (ii) selenium and health (including the effect of intake and/or status biomarkers on cancer risk, immune function, HIV, cognition, and fertility), (iii) bioavailability of selenium from the diet, and (iv) impact of genotype/single nucleotide polymorphisms on status or health outcomes associated with selenium. The main research outputs for selenium and future research priorities are discussed further in this review.

Keywords Selenium, nutrition, dietary reference values, selenoprotein P, glutathione peroxidase, plasma selenium, serum selenium, requirements

DIETARY SOURCES, ABSORPTION AND METABOLISM, AND FUNCTIONS OF SELENIUM

The main dietary sources of the essential micronutrient selenium include fish, shellfish, cereals, meat, and dairy products, however the precise content in foods can vary greatly depending on where the plants/animals are grown or produced. Various forms of selenium are present in foods including predominantly selenomethionine or selenocysteine plus other forms such as, selenocystine, Se-methylselenocysteine, γ-glutamyl-Se-methylselenocysteine, selenocystathionine, selenoneine, selenite, or selenate. The forms depend on the type of food and the selenium concentration (Infante et al., 2005; Dumont et al., 2006; Rayman, 2008, 2012). The profile (or relative contribution) of different forms of selenium in any one food can be stable, for example in wheat where the predominant form is selenomethionine ($\sim$65–85%), with smaller amounts of selenocysteine, Se-methylselenocysteine, selenite, and selenate, which are present over a wide range of total selenium concentration (Wolf and Goldschmidt, 2007; Cubadda et al., 2010; Hart et al., 2011). However in some foods, for example broccoli, garlic, and onions (plants that are selenium accumulators), the relative contribution of the different forms of selenium will vary depending on the level of enrichment or selenium concentration (Kotrebai et al., 2000). The complex profile of selenium forms in foods consumed impacts on the absorption and metabolism of selenium—discussed in section on bioavailability below and in reviews (Rayman, 2008; Fairweather-Tait et al., 2011).

The function of selenium is partly through its role via incorporation into selenoproteins of which there are 25 known selenoprotein genes (Kryukov et al., 2003). Selenoprotein P (Sepp1) accounts for >25% up to 50% of selenium in plasma (Deagen et al., 1993; Akesson et al., 1994; Burk et al., 2001) and is the main transport protein for selenium (Burk and Hill, 2009). Other selenoproteins include the glutathione peroxidase family (GPx 1–4 & 6), thioredoxin reductases (TrxR1-3), selenoproteins: SelH, SelI SelK, SelM, SelN, SelO, SelR, SelS, SelT, SelV, SelW, the 15 KDa selenoprotein, selenophosphate synthetase (SPS2), and the iodothyronine deiodinases (Dio1-3) (reviewed recently in Bellinger et al., 2009; Lu and Holmgren,

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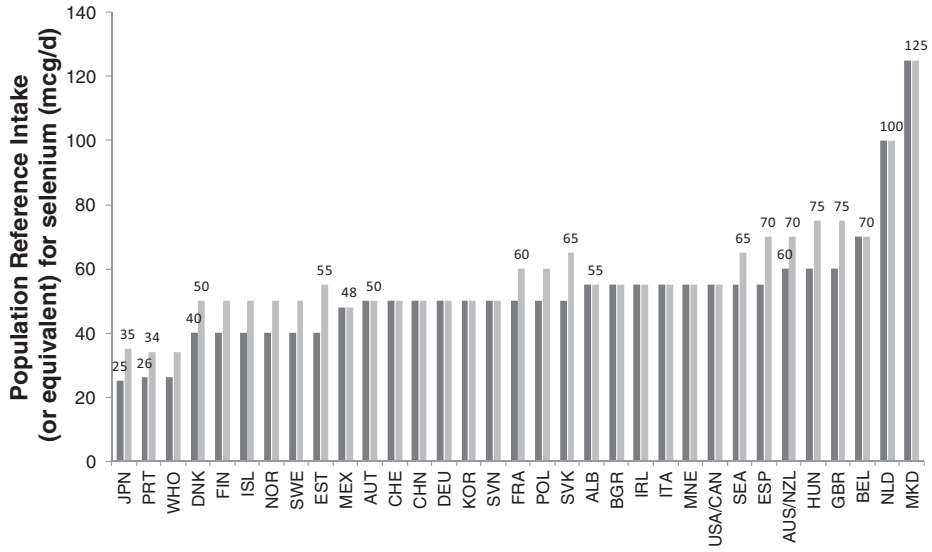

Figure 1 Selenium recommendations from different countries/expert bodies. Data presented in the figure obtained from online EURRECA web resource: http://www.serbianfood.info/eurreca/. The black and gray bars indicate recommendations for female and male adults, respectively (Cavelaars et al., 2010). *Abbreviations: JPN, Japan; PRT, Portugal; WHO, World Health Organisation; DNK, Denmark; FIN, Finland; ISL, Iceland; NOR, Norway; SWE, Sweden; EST, Estonia; MEX, Mexico; AUT, Austria; CHE, Switzerland; CHN, China; DEU, Germany; KOR, South Korea; SVN, Slovenia; FRA, France; POL, Poland; SVK, Slovakia; ALB, Albania; BGR, Bulgaria; IRL, Ireland; ITA, Italy; MNE, Montenegro; USA/CAN, United States of America/Canada; SEA, South East Asia region; ESP, Spain; AUS/NZL, Australia and New Zealand; HUN, Hungary; GBR, United Kingdom; BEL, Belgium; NLD, Netherlands; MKD, Macedonia.*

2009; Steinbrenner and Sies, 2009; Fairweather-Tait et al., 2011; McCann and Ames, 2011). The selenoproteins have a wide range of functions including protection against lipid and protein damage, antioxidant protection, redox signaling, maintaining muscle fibers, thyroid hormone homeostasis and selenium homeostasis (Bellinger et al., 2009; Burk and Hill, 2009; Lu and Holmgren, 2009; Fairweather-Tait et al., 2011; McCann and Ames, 2011; Schweizer et al., 2011; Rayman, 2012).

Selenium is required for optimal function of the thyroid, testis, prostate, brain, and for muscle development and function (Lescure et al., 2008, 2009; Fairweather-Tait et al., 2011; Schweizer et al., 2011; Berr et al., 2012; Rayman, 2012). Associations between selenium and immune function and various other health outcomes such as fertility, cognitive function, cardiovascular disease, osteoarthritis, type-2 diabetes, cancer risk, and mortality have been reported and are the focus of recent review papers (Fairweather-Tait et al., 2011; Rayman, 2012). The evidence for the association between selenium intake and status and several of the health outcomes was reviewed as part of the EURRECA Network of Excellence Research Activities. Where sufficient data and evidence were available systematic reviews and meta-analyses were completed and are summarized below.

CURRENT DIETARY RECOMMENDATIONS

Current dietary reference values for adults in most countries are based on the intake of selenium required to achieve maximal glutathione peroxidase activity in plasma or erythrocytes. There are variations across Europe and worldwide, ranging from 25 μg/day for adult women in Japan up to $\geq$ 100 μg/day in the Netherlands and Macedonia (Figure 1).

The dietary reference values from selected sources for all population groups including infants, children, adolescents, adults, elderly, pregnant, and lactating women are shown in Table 1. For infants aged 0–6 months several countries/expert bodies use the intake estimated from breast milk data (e.g., Department of Health, 1991; Institute of Medicine, 2000; Otten et al., 2006) to calculate adequate intake and the reference values. For children and adolescents, the data are usually extrapolated from the adult reference values, accounting for differences in body weight. To account for increases during pregnancy and lactation an additional 5–20 μg selenium per day is added to the reference value (Table 1). For nearly all countries/expert bodies the elderly (>65 years) have the same reference values as adults as there is no clear evidence to suggest that they have an increased requirement. As identified in a EURRECA workshop with micronutrient experts in Leiden, Netherlands (EURRECA Network and Expert working group, 2011) the main population group where selenium requirements may need further review are adolescent males as discussed further (see Research gaps and Priorities).

CURRENT INTAKES/ADEQUACY

The intake of selenium varies in different countries. Europe is generally considered to have relatively low intakes, with the average intakes of several countries falling below the reference values (Rayman, 2008; Flynn et al., 2009; Fairweather-Tait et al., 2011). Intakes calculated from several European countries

Table 1 Selected[†] dietary reference values for selenium intake (mcg/d)

Data source/year	Gender	Population group							Upper level of safe intake (adults)
		Infants	Children	Adolescents	Adults	Elderly	Lactation	Pregnancy	
WHO/FAO 1998/2004	Male	*0–6m* 6 *7–12m* 10	*1–3y* 17 *4–6y* 22 *7–9y* 21	*10–18y* 32	*19–65y* 34	*>65y* 33	–	–	400
	Female	*0–6m* 6 *7–12m* 10	*1–3y* 17 *4–6y* 22 *7–9y* 21	*10–18y* 26	*19–65y* 26	*>65y* 25	*0–6m pp** 35 *7–12m pp** 42	*2nd trimester* 28 *3rd trimester* 30	400
Nordic/2004	Male	*6–11m* 15	*1–2y* 20 *2–5y* 25 6–9y 30 *10–13y* 40	*14–17y* 50	*18–30y* 50 *31–60y* 50 *61–74y* 50	*≥75y* 50	–	–	300
	Female	*6–11m* 15	*1–2y* 20 *2–5y* 25 6–9y 30 *10–13y* 40	*14–17y* 40	*18–30y* 40 *31–60y* 40 *61–74y* 40	*≥75y* 40	55	55	300
Australia/ NZ/2005	Male	*0–6m* 12 *7–12m* 15	*1–3y* 25 *4–8y* 30 *9–13y* 50	*14–18y* 70	*19–30y* 70 *31–50y* 70 *51–70y* 70	*>70y* 70	–	–	400
	Female	*0–6m* 12 *7–12m* 15	*1–3y* 25 *4–8y* 30 *9–13y* 50	*14–18y* 60	*19–30y* 60 *31–50y* 60 *51–70y* 60	*>70y* 60	*75*	65	400
Japan/2005	Male	*0–5m* 16 *6–11m* 19	*1–2y* 9 *3–5y* 10 *6–9y* 15 *10–11y* 20	*12–14y 25* *15–17y* 30	*18–29y* 30 *30–49y* 35 *50–69y* 30	*≥70y* 30	–	–	450
	Female	*0–5m* 16 *6–11m* 19	*1–2y* 8 *3–5y* 10 *6–9y* 15 *10–11y* 20	*12–14y 25* *15–17y* 25	*18–29y* 25 *30–49y* 25 *50–69y* 25	*≥70y* 25	+20 μg additional	+4 μg additional	350
EC/1993	Male	*6–11m* 8	*1–3y* 10 *4–6y* 15 *7–10y* 25	*11–14y* 35 *15–17y* 45	*≥18y* 55	–	–	–	300[#]
	Female	*6–11m* 8	*1–3y* 10 *4–6y* 15 *7–10y* 25	*11–14y* 35 *15–17y* 45	*≥18y* 55	–	70	55	300[#]
FESNAD/2010	Male	*0–6m* 10 *7–12m* 15	*1–3y* 20 *4–5y* 20 *6–9y* 25	*10–13y* 35 *14–19y* 50	*20–69y* 55	*>70y 55*	–	–	–
	Female	*0–6m* 10 *7–12m* 15	*1–3y* 20 *4–5y* 20 *6–9y* 25	*10–13y* 35 *14–19y* 45	*20–69y* 55	*>70y 55*	70	55	–
UK/1991	Male	*0–3m* 10 *4–6m* 13 *7–12m* 10	*1–3y* 15 *4–6y* 20 *7–10y* 30	*11–14y* 45 *15–18y* 70	*19–50y* 75	*>50y 75*	–	–	450[#]
	Female	*0–3m* 10 *4–6m* 13 *7–12m* 10	*1–3y* 15 *4–6y* 20 *7–10y* 30	*11–14y* 45 *15–18y* 60	*19–50y* 60	*>50y* 60	+ 15 μg additional	–	450[#]
Institute of Medicine (US, Canada)/2000	Male	*0–6m* 15 *7–12m* 20	*1–3y* 20 *4–8y* 30 *9–13y* 40	*14–18y* 55	*19–30y* 55 *31–50y* 55 *51–70y* 55	*>70y* 55	–	–	400
	Female	*0–6m 15* *7–12m 20*	*1–3y 20* *4–8y 30* *9–13y 40*	*14–18y 55*	*19–30y 55* *31–50y 55* *51–70y 55*	*>70y 55*	*70*	*60*	*400*

Data presented in Table 1 was obtained from online EURRECA web resource *NutriRecQuest*: http://www.serbianfood.info/eurreca/—(Cavelaars et al., 2010) and original source documents (Federacion Espanola de Sociedades de Nutricion Alimentacion y Dietetica [FESNAD], 2010; Institute of Medicine, 2000; Department of Health, 1991; Scientific Committee for Food, 1993; Nordic Council of Ministers, 2004; World Health Organization, 2004; Ministry of Health Labour and Welfare Japan, 2005; Australian Government Department of Health and Aging New Zealand Ministry of Health and National Health and Medical Research Council, 2005; Scientific Committee for Food, 1993; Nordic Council of Ministers, 2004; World Health Organization, 2004; Ministry of Health Labour and Welfare Japan, 2005; Australian Government Department of Health and Aging New Zealand Ministry of Health and National Health and Medical Research Council, 2005; Otten et al., 2006).

[†]RDA, Recommended Dietary Allowance (USA, Japan); equivalent to RNI, Reference Nutrient Intake (UK); PRI, Population Reference Intake (EFSA); RNI, Recommended Nutrient Intake (WHO/FAO); RDI, Recommended Dietary Intake (AU/NZ); RI, Recommended Intake (Nordic).

[*]pp = postpartum.

[#]Safe upper limit of selenium intake for UK from (Food Standards Agency Expert Group on Vitamins and Minerals, 2003); Upper level of safe intake for European Community from (European Food Safety Authority, 2006).

including Denmark, Finland, Italy, Poland, UK, and The Netherlands ranged from mean intakes of 34–67 μg/day for adult women and 43–85 μg/day for adult men (Flynn et al., 2009). Finland has the highest range of intake compared to other European countries due to the mandatory use of selenium fertilizers since 1985 (Alfthan et al., 1991). Risk of excessive intake of selenium in Europe is considered relatively low, even when taking into account Se-fortified foods and selenium supplement intake (Flynn et al., 2009).

As part of the EURRECA research activities the prevalence of inadequate intakes in Europe for several nutrients, including selenium, were calculated and estimated using published data from 13 EU countries (Roman Vinas et al., 2011). Where data were available for selenium (from five countries), in general there was a relatively high prevalence of inadequate intake across Europe, with >20% of the included populations estimated to be at risk of inadequacy, based on the percentage of the population below the AR of the Nordic Nutritional Recommendations (Roman Vinas et al., 2011). There were country and region specific variations in the selenium intakes and therefore in the prevalence of estimated inadequate intake; for example, estimates of inadequacy ranged from 8–11% in adult men/women in the Netherlands and Finland and up to 30–47% in Denmark and Sweden (Roman Vinas et al., 2011).

In order to make regional comparison in micronutrient intake/status and risk of inadequacy in Europe data were collected for all population groups in Central and Eastern Europe (CEE), Scandinavia, Western Europe and Mediterranean countries. Studies were collected from electronic databases (PubMed, Embase) and from gray literature sources. The inclusion criteria followed general EURRECA guidelines on dietary intake and study characteristics (see Activities 1 and 3 in Dhonukshe-Rutten et al., 2013). However, regional comparison of selenium intake was not possible due to lack of studies from CEE (Novakovic et al., 2012a). To evaluate the association between socioeconomic (SES) determinants of micronutrient intake and status in Europe, a systematic literature search was performed using Medline and Embase databases. Studies were considered eligible if they followed Best Practice Methods (see Activities 1 and 3 in Dhonukshe-Rutten et al., 2013) for assessment of micronutrient intake and/or status. Data on selenium, which were reported by one study of Irish adults and one study in UK children, showed a slightly lower mean intake in the low SES group for intake by education and occupation. Relative differences between the SES groups were less than 10% and not consistent for the different SES indicators. The mean selenium intakes observed for both studies and SES groups were above the reference values for the average requirement (Novakovic et al., 2012b).

In a recent overview by Mensink et al. (2013) on low intakes of micronutrients across Europe, the percentage of children aged 1–3 years with intakes below the Nordic Nutritional Recommendations AR were 3% in the UK and 25% in Belgium. For girls aged 11–17 years 54% were below the AR in France and 68% in the Netherlands, and for boys, 38% were below the AR in France and 52% in the Netherlands. In adults, the prevalence of selenium intakes below the AR was high in all countries, e.g., 55% of women in France and 75% in the Netherlands, and 59% of men in France and 63% in the UK.

ESTABLISHING THE MOST ROBUST METHODOLOGY

Intake Assessment

In a series of EURRECA systematic reviews focusing on the assessment and validation of different methods to estimate intake, the evidence for selenium intake estimates in the adult, elderly, and pregnant women populations were evaluated (for a summary of methodology see Activity 3 in Dhonukshe-Rutten et al., 2013). For adults and elderly, overall estimation of selenium intake using food frequency questionnaire (FFQ) methods showed acceptable correlations with other methods of validation including dietary records and 24 hour recalls (reviewed in Serra-Majem et al., 2009; Ortiz-Andrellucchi et al., 2009b). Only one identified study (Karita et al., 2003) validated FFQ and dietary records with biomarkers (erythrocyte/serum selenium concentration). Serum selenium did not correlate with dietary intake in the included study (Karita et al., 2003; Serra-Majem et al., 2009), this is most likely due to the form of selenium consumed since plasma/serum selenium is a sensitive marker for selenomethionine content of food (Burk et al., 2006) or Se-enriched yeast supplement (Hurst et al., 2010) but not other forms of selenium (Levander et al., 1983; Thomson and Robinson, 1986; Alfthan et al., 1991; Thomson et al., 1993; Xia et al., 2005; Burk et al., 2006; Hurst et al., 2010). Also, the relationships between intake and selenium status biomarkers are not linear due to the plateau of several markers (e.g., SEPP1, GPx1, and GPx3) at Se-replete intakes >80–100 μg/day (Levander et al., 1983; Neve, 1995; Duffield et al., 1999; Burk et al., 2006; Hurst et al., 2010), which makes validation assessment methods more complex. For example, in a recent study of a Se-replete population from the USA, consuming on an average 109 $\pm$ 43 μg/day selenium, the intake estimated by FFQ did not correlate with the status biomarkers analyzed—GPx3, plasma/buccal/urine selenium, SEPP1 (Combs et al., 2011).

For dietary assessment methods to estimate selenium intake in pregnant women, two validation studies (Erkkola et al., 2001; Mouratidou et al., 2006) were identified in a systematic review by (Ortiz-Andrellucchi et al., 2009a). Overall, the FFQ estimated a higher selenium intake than a 24 hour recall or food records. There were no published studies identified for the validation of methods to assess selenium intake in pregnant women with comparison to known selenium status biomarkers (Ortiz-Andrellucchi et al., 2009a).

In summary, it is difficult to obtain accurate intake estimates for habitual intakes of selenium. Food records, diaries, and questionnaires to assess intake are of limited value due to problems with the high variation in selenium content depending on where the food was grown and therefore potential inaccuracies arising from composition tables for the selenium

content of food (Fairweather-Tait et al., 2011). The different forms of selenium in foods, of which there are usually more than three different forms of selenium (predominantly selenomethionine and selenocysteine), add further complexity with respect to intake data. Estimating the intake of selenium may also be difficult when there are individuals who take supplements.

In order to accurately assess the relationship between intake and status biomarkers and/or health outcomes the use of intake data from high-quality studies (e.g., randomized controlled trials), especially from selenium supplementation studies comparing different forms of selenium, is more informative as the effects of selenium have been shown to be form-specific (discussed further below).

Status Assessment

In a systematic review of human selenium status biomarkers using the EURRECA criteria for evidence and study data assessment (Ashton et al., 2009; Hooper et al., 2009), the following selenium status biomarkers were considered to be a useful reflection of intake: selenium concentration in plasma, erythrocyte or whole blood, plasma selenoprotein P and glutathione peroxidase activity (quantified in plasma, platelet or whole blood). There were insufficient data to complete the review of evidence for other biomarkers including toenail selenium, and muscle or erythrocyte GPx activity (Ashton et al., 2009). A set of best practice guidelines with relevant information on selenium biomarkers, plus a table of quality rating for each of the biomarkers of selenium status were produced in the EURRECA Network of Excellence with expert consultation (Harvey et al., 2011).

Several selenium status biomarkers were reported to be sensitive over the range of deficiency to repletion, including plasma or erythrocyte glutathione peroxidase (GPx1 and 3) activity and plasma selenoprotein P (Sepp1) (Thomson, 2004; Burk et al., 2006; Ashton et al., 2009). Above intakes of 60–100 μg/day the status biomarkers GPx1, GPx3, and Sepp1 approach maximum expression levels and do not accurately reflect selenium intake or status above 110 μg/day (Burk et al., 2006). GPx1 and GPx3 activities reach maximal activity at intakes below 70 μg/day (Levander et al., 1983; Thomson et al., 1993; Duffield et al., 1999), below the range associated with several health benefits of selenium—reviewed later; whereas, Sepp1 is maximal at higher selenium intakes ($\sim$100 μg/day; Duffield et al., 1999; Burk et al., 2006; Hurst et al., 2010; Xia et al., 2010). The reliable, sensitive selenium status biomarkers for selenium deficient or replete populations, including Sepp1 and plasma selenium, are discussed further. For populations with intakes above the Se-replete range more informative functional biomarkers of selenium status are required. Toenail selenium is a useful longer term biomarker of status and reflects tissue selenium levels over a relatively wide range of intake (Longnecker et al., 1993; Longnecker et al., 1996; Behne et al., 2010). Toenail selenium was included in the EURRECA systematic reviews on selenium status and health (in particular prostate cancer risk, summarized below). Methodology to analyze a combination of biomarkers may also be useful (Elsom et al., 2006; Hoeflich et al., 2010), for example the calculation of the fraction of plasma selenium attributed to GPx3, Sepp1, and other selenium, labeled "other selenium" (Burk et al., 2006) or "non-specific selenium" (Combs et al., 2011), which may account for between 30–50% of total selenium in plasma (Burk et al., 2006; Ballihaut et al., 2011; Combs et al., 2011). This may provide informative status biomarkers for populations where selenium intakes are relatively high, above the level required to reach maximal Sepp1 and GPx3.

Identification of Selenium Intake/Status Biomarkers Using Metabolomics

To date there are limited data on the global metabolite profile in plasma in relation to selenium intake and/or status, and as part of the EURRECA network research activity, the aim was to identify markers of selenium intake using state-of-the-art metabolomics methodology (Bayle et al., 2012). The development of the metabolomics platform was based on three micronutrient "biological networks" including selenium. The selection of markers that were covered by the platform included markers of micronutrient exposure, target function, biological response, and selenium-related health and disease parameters. Advancing the development of the platform with further biological knowledge may increase the chance of finding relevant markers. The application of metabolomics to the selenium research area presents future possibilities for novel intake and/or status biomarker identification and functional status-health profile markers. There are few studies on selenium and "global" metabolomics, a majority of the work to date has focused on identification and quantification of targeted selenium metabolites in cell culture, animal model, or human urine samples. Major urinary selenium metabolites in both rats and humans were identified to be monomethylated selenium, 1β-methylseleno-N-acetyl-D-galactosamine (selenosugar), trimethylselenonium (Kobayashi et al., 2002; Suzuki, 2005; Ohta et al., 2009), and other selenosugars (Gammelgaard and Bendahl, 2004; Kuehnelt et al., 2005; Letsiou et al., 2007). The ratio of selenosugar and trimethylselenonium metabolites in urine were determined to be responsive to selenium dose in rats up to low toxicity range (Kobayashi et al., 2002; Suzuki et al., 2005) and there is an indication that the urine selenium metabolite profile alters in response to high doses of selenium, or indeed different forms of selenium (Kuehnelt et al., 2005; Suzuki, 2005; Kuehnelt et al., 2007; Ohta et al., 2009). Se-methylselenoneine has recently been identified as a novel urinary metabolite (Klein et al., 2011), and methods to quantify volatile urine selenium species have been identified (Bueno and Pannier, 2009), thus making further profiling of targeted selenium metabolite analysis an interesting area for future research.

Molecular Markers of Selenium Status

Inclusion of molecular markers in the EURRECA systematic review on biomarkers of selenium status (Ashton et al., 2009)

was not possible due to lack of data up to 2007. Since then, several potential molecular markers of selenium status have been assessed including GPX1, GPX3, GPX4, SELH, SEPW1, SEPP1 mRNA levels in whole blood, lymphocytes or peripheral blood mononuclear cells (Pagmantidis et al., 2008; Sunde et al., 2008; Goldson et al., 2011). In a longitudinal study with an average study population intake of 48 $\pm$ 14 μg/day and plasma selenium concentration of 1.13 $\pm$ 0.16 μmol/L none of the molecular markers assessed in whole blood total RNA (GPX1, GPX3, GPX4, SEPP1, SEPW1, SELH) correlated with plasma selenium. The authors concluded that molecular markers of selenium status should be evaluated in selenium deficient populations. In a selenium supplementation study, with 100 μg/day sodium selenite over 6 weeks, SEP15 and SELK were Se-responsive and warrant further investigation as potential molecular markers of selenium status (Pagmantidis et al., 2008). Also, SEPW1 gene expression levels were significantly different after 200 μg/day Se-enriched yeast and 50 μg/day Se-enriched onion meals when compared to a placebo group (Goldson et al., 2011). However, to fully evaluate molecular markers of selenium status further research and validation of the biomarkers would be required.

In summary, the systematic review process employed in EURRECA has highlighted several useful biomarkers of selenium status including selenoprotein P, plasma/serum selenium, and toenail selenium (Ashton et al., 2009; Harvey et al., 2011). Comparison of the validated status biomarkers with health outcomes was the focus of further systematic reviews and the summary evidence presented below.

Relevant Health Outcomes

The health outcomes selected for further investigation as part of the EURRECA research network were prioritized through a combination of basic database searches (to establish the volume of publications for each topic), an assessment of systematic reviews already in existence (to prevent repetition of work), a directive to include vulnerable groups and an eminence-based assessment of the most relevant and important outcomes.

Potentially relevant systematic reviews were found through the main search results and by checking reference lists of other reviews. Each review was assessed for relevance and quality according to a series of criteria (Critical Appraisal Skills Programme [CASP] guidelines). Following this process, the EURRECA prioritized health outcomes for selenium were (i) cognition assessed by mini mental state examination (MMSE), memory recall or other cognitive test score in populations >50 yrs, (ii) viral load and markers of infection in HIV patients (a vulnerable group), including data from CD4 cell count, CD8 cell count, CD4/8 ratio, HIV viral load, or HIV-1 RNA, (iii) immunity assessed by number/rate or duration of infection or antibody titers, (iv) male fertility including assessment of sperm count, morphology and/or motility data (Collings et al., 2009; Collings et al., 2012), and (v) cancer, in particular the dose-response relationship between selenium and total and advanced prostate cancer risk. A summary of the EURRECA systematic review outputs on selenium intake/status and health are presented ahead.

Population Groups

The EURRECA systematic review and meta-analyses of relevant studies on selenium focused on data from adult and elderly populations. The EURRECA work on selenium was carried out in addition to the five prioritized micronutrients (iron, folate, B12, iodine, and zinc), and with a particular focus on interactions with vitamin E and requirements for vulnerable groups. The principal health outcomes of interest were most relevant in these population groups, and it was predicted that the vast majority of data would be available in adult populations.

As part of the expert consultation process in EURRECA, young adolescent males were identified as a population group requiring further consideration due to the selenium demand required for testes and prostate function during puberty. Future work to generate high-quality data for the calculation of selenium requirements for adolescent males was recommended (discussed in "Research gaps and priorities" later).

COLLATING, SUMMARIZING, AND INTERPRETING SOURCES OF EVIDENCE

Factorial/Bioavailability Approach

The factorial approach, as used to derive dietary requirements for iron or zinc, for example, (Harvey et al., 2013; Lowe et al., 2013) is less relevant for selenium as valid data are available to assess the dose response relationship between intake, status, and health. Using the dose-response approach is particularly important due to the potential narrow range for beneficial effects of selenium (reviewed in Fairweather-Tait et al., 2011; Rayman, 2012). The bioavailability of selenium, is generally high: >90% of selenomethionine is absorbed, selenocysteine appears to be absorbed very well; ~100% of selenate is absorbed but a significant fraction is lost in the urine; and >50% of selenite is absorbed (depending on luminal interactions) and it is better retained than selenate (Thomson and Robinson, 1986; Institute of Medicine, 2000; Fairweather-Tait et al., 2010). Due to the complex forms of selenium present in foods, it is very difficult to estimate bioavailability from whole diets. Estimation of the absorption and bioavailability of selenium forms from the diet also depends on methods used to assess absorption and bioavailability, for example if plasma selenium is used (e.g., Bugel et al., 2001; Hawkes et al., 2003; Kirby et al., 2008; Thomson et al., 2008) this may not reflect the "true" bioavailability of all forms of selenium in the diet, since plasma selenium only reflects selenomethionine intake (Burk et al., 2006). Also, if plasma (GPx3)/whole blood/platelet or erythrocyte glutathione

peroxidase (GPx1) are used (e.g., Hawkes et al., 2003; Thomson et al., 2008), GPx1 and 3 are prone to fluctuations due to other dietary or health factors (discussed ahead) and the activity plateaus (Thomson et al., 1982; Neve, 1995; Duffield et al., 1999; Xia et al., 2005). Hence, this may result in an over- or underestimate of bioavailability. The highest quality data for selenium bioavailability are from selenium stable isotope studies (Fairweather-Tait, 1997; Finley, 2006; Fairweather-Tait et al., 2010) discussed further in an eminence based review on selenium bioavailability conducted in EURRECA (Fairweather-Tait et al., 2010); stable isotope bioavailability studies demonstrated for several forms of selenium, that absorption was relatively high, e.g., 89% in response to ^{77}Se-yeast (Bugel et al., 2008). The data on bioavailability of selenium from single meal/food intervention studies were reviewed using systematic techniques and protocol as part of EURRECA research activities and summarized in (Collings et al., 2010).

Intake-status-health Relationships

Intake-status Relationships

There are well defined intake-status (I-S) relationships for key selenium biomarkers in several populations; data are in good agreement for glutathione peroxidase 1 and 3, plasma/serum selenium and selenoprotein P (Ashton et al., 2009; EURRECA Network and Working Party on Status Biomarkers, 2011; Harvey et al., 2011). Since the EURRECA systematic review was undertaken to assess the usefulness of several selenium biomarkers (Ashton et al., 2009), new evidence on the association between intake and status for two of the main biomarkers (Sepp1, plasma/serum selenium) became available for evaluation and is summarized in this review. An up-to-date review on the usefulness of plasma GPx3 activity data (used for current dietary selenium recommendations) is also included, together with recent data on toenail selenium as a biomarker of intake and tissue status.

- **Glutathione peroxidase (GPx) activity in plasma (GPx3), erythrocytes, whole blood or lymphocytes (GPx1).** GPx1 and plasma GPx3 are useful biomarkers of intake up to ~50–70 μg/day. Current dietary recommendations for selenium intake are based on the intake required for maximal GPx3 or erythrocyte GPx1 activity (see section above). However, there is evidence of a variable response in plasma GPx3 and erythrocyte GPx1 related to different forms of selenium (Thomson et al., 1982; Xia et al., 2005). Also, levels of GPx1 and 3 can be altered in certain disease states (Gartner et al., 2001; Lee et al., 2005; Schnabel et al., 2005; Zachara et al., 2006; Arsova-Sarafinovska et al., 2009; Cheng et al., 2009; Agnani et al., 2011; Guo et al., 2011; McCann and Ames, 2011) or due to certain polymorphisms (Ozata et al., 2000; Reszka et al., 2007; Lei et al., 2009; Malling et al., 2009; Schomburg et al., 2009; Giacconi et al., 2010; Xiong et al., 2010). This complicates interpretation of GPx1 and 3 data in terms of relationship with selenium intake/health. Further, the maximal level of GPx 1 and 3 activity is achieved below the range of selenium intake and corresponding selenium status where reduction in risk observed (Akbaraly et al., 2005; Bleys et al., 2008; Fairweather-Tait et al., 2011; Hurst et al., 2012; Rayman, 2012).
- **Plasma/serum selenium concentration**. Plasma/serum selenium responds over a wide range of intakes, although predominantly reflects intake of one particular form of selenium, namely selenomethionine (and is not as responsive to other forms of selenium in Se-replete populations). For example, plasma selenium increases and responds to intake of selenomethionine, or selenomethionine rich food/supplement including Se-enriched yeast, Se-enriched wheat (Levander et al., 1983; Alfthan et al., 1991; Burk et al., 2006; Hurst et al., 2010; Combs et al., 2012) but does not show a similar increase in response to selenate (Levander et al., 1983; Alfthan et al., 1991), selenite (Alfthan et al., 1991; Burk et al., 2006), or Se-enriched onion meals (Hurst et al., 2010). In a selenomethionine dose response RCT from the US, with 50–200 μg selenomethionine supplement per day on top of the habitual intake (109 μg/day) (Combs et al., 2011) plasma selenium concentration was linearly related to selenomethionine dose (Combs et al., 2012). Yang et al. (1989) also defined the response of plasma selenium to selenium intake. Several similar equations have been published to estimate intake of selenomethionine from plasma selenium data from several populations (e.g., Burk et al., 2006), the relationship between intake and plasma selenium is well described and consistent. The length of time taken to reach maximal expression level or steady state concentration with a range of selenium intakes is also predictable from published data. For example, plasma/serum selenium concentration will reach a steady state after 10–12 weeks in a selenium deficient or adequate population and moderate doses of selenium (~100–200 μg/day) (Thomson et al., 1982; Alfthan et al., 1991; Neve, 1995; Duffield et al., 1999; Xia et al., 2005; Burk et al., 2006; Combs et al., 2009); however, in a Se-replete population (plasma selenium >130 ng/ml), plasma/serum selenium concentration may require longer duration (~36 weeks) to reach steady state with supplement doses $\geq$ 100 μg/day selenomethionine (Combs et al., 2012).
- **Selenoprotein P concentration in plasma.** Sepp1 is one of five essential selenoproteins (McCann and Ames, 2011). Plasma selenoprotein P levels respond to different forms of selenium in the diet in selenium deficient/replete populations (Persson-Moschos et al., 1998; Duffield et al., 1999; Xia et al., 2005; Hurst et al., 2010; Xia et al., 2010).

Since Sepp1 was identified as a useful biomarker of selenium intake in the EURRECA systematic review (Ashton et al., 2009), several new high-quality studies have been published. An update of relevant data from studies investigating the dose and form of selenium required to maximize selenoprotein P in plasma is

presented below, including randomized controlled trial data (Table 2). In summary several RCTs are in agreement that plasma selenoprotein P responds to intake of different forms of selenium and intake in selenium deficient/replete populations (Xia et al., 2005; Hurst et al., 2010; Xia et al., 2010). Data from New Zealand, China, UK and US are in agreement and several selenium experts consider plasma selenoprotein P maximal levels as a suitable status biomarker for calculating recommendations for selenium intake (Burk and Hill, 2005; Xia et al., 2005; Burk et al., 2006; Rayman, 2008; Hurst et al., 2010; Fairweather-Tait et al., 2011). Depending on baseline selenium status, Sepp1 may reach steady state levels after a supplementation period of 10 weeks (Persson-Moschos et al., 1998; Xia et al., 2005; Hurst et al., 2010), or if in severely selenium deficient population, such as in China where intakes were estimated to be 14 μg/day, longer supplementation periods may be required to achieve maximum Sepp1 (Xia et al., 2010) (data compared in Table 2).

To take into account recent evidence that has emerged from high-quality RCTs, and to further evaluate the dose response relationship between selenium intake and Sepp1 or plasma selenium concentration in different populations, analysis of individual participant data from RCTs (where available) may be informative.

Selenium intake-health and Status-health Relationships

Selenium intake and/or status have been associated with many health outcomes (reviewed recently in Fairweather-Tait et al., 2011; Rayman, 2012). As part of the EURRECA research activities the data to assess the effect of varying selenium intakes and/or status were reviewed. For instance, relations between selenium intake or status and inflammatory, oxidative, and metabolic markers were reviewed. This information can be found in NuGOwiki (http://nugowiki.org/index.php/Selenium). In addition, the effect of varying selenium intakes and/or status on male fertility; viral load and markers of infection in HIV+ patients, and infection, immunity; and cognition in the elderly were systematically reviewed according to protocol (Collings et al., 2009), with methodology as detailed in Collings et al. (2012). For the systematic review on selenium and prostate cancer the protocol used was as referred to in Hurst et al. (2012). Dose-response meta-analyses, with restricted cubic splines and fractional polynomials for nonlinear trends, were used to investigate the relationship between selenium status and prostate cancer risk (Hurst et al., 2012). The main data sources and evidence reviewed are summarized ahead.

Selenium and Cognition

The effect of selenium nutrition (and/or vitamin E) on the rate of cognitive decline in the older population was assessed (Collings et al., 2012). Papers were collected, which reported the effects of selenium (and/or vitamin E) on cognitive decline in adults aged over 50 years. In total, 44 full text papers were collected and screened, and four studies were included (comprising six papers). The low number of included studies, i.e., only three RCTs (Clausen et al., 1989; Rayman et al., 2006; Gosney et al., 2008) and one cohort (Akbaraly et al., 2007), meant that meaningful meta-analysis was not possible and the RCTs were summarized. Rayman et al. (2006) reported no significant improvement in cognition (as assessed by mood scores and quality of life scores) following supplementation with selenium alone at three different dose levels, despite reporting a significant increase in selenium status in the intervention groups compared to placebo. However, both multinutrient intervention studies (Clausen et al., 1989; Gosney et al., 2008) reported a significant improvement in psychological and depression scores in the intervention groups (300 μg selenomethionine + 465 mg vitamin E (Clausen et al., 1989), and 60 μg selenium + 60 mg vitamin E (Gosney et al., 2008) compared to placebo.

Selenium and HIV

One of the goals of EURRECA was to investigate the micronutrient requirements of vulnerable Europeans, and therefore HIV positive individuals were included as a vulnerable group. The effect of selenium supplement on health outcomes in HIV patients was assessed by systematic review using modified standardized EURRECA methodology (Collings et al., 2009; Collings et al., 2012). Fourteen studies met the inclusion criteria detailed in 16 papers; all but two included studies were RCTs. Of the 14 included studies, six were conducted in African countries (Kupka et al., 2004; McClelland et al., 2004; Range et al., 2006; Semba et al., 2007; Kupka et al., 2008; Villamor et al., 2008), five in the USA (Baum et al., 1997a; Baum et al., 1997b; Burbano et al., 2002; Shor-Posner et al., 2003; Kaiser et al., 2006; Hurwitz et al., 2007), two in Europe (Constans et al., 1996; Look et al., 1998), and one in Asia (Jiamton et al., 2003). Of the 12 studies administering an intervention, five gave only a selenium supplement and seven gave selenium as part of a multiple micronutrient supplement or in addition to one or more other micronutrient supplements. The amount of selenium provided varied between studies from 65 μg/d (Semba et al., 2007) to 500 μg/d (Look et al., 1998). Duration also varied between the studies, from 6 weeks (McClelland et al., 2004) to 24 months (Semba et al., 2007; Villamor et al., 2008). No studies were deemed to be of low risk of bias (Collings et al., 2012). With these caveats in mind, the meta-analysis of data on selenium supplementation and post-intervention CD4 count in eight of the relevant included studies representing data from 2231 participants, showed that there was no significant effect of selenium supplementation intervention on CD4 count post-intervention in HIV patients (WMD: 2.64; 95% CI: -16.93, 22.22; $p = 0.79$; $I^2 = 0\%$) (forest plot shown in Collings et al., 2012). However, further investigation taking into account baseline status with analysis of data on selenium supplementation and change in CD4 count from baseline representing data from 676 participants, and four studies indicated a significant effect of selenium on CD4 count change from baseline (WMD: 36.45; 95% CI: 0.56, 72.33; $p = 0.05$), with significant heterogeneity

Table 2 Summary of selenium intake and plasma selenium concentration required for plasma selenoprotein P maximal expression from randomised controlled trials (RCTs) and human intervention studies with healthy adults

Study details: study type, duration, dose of selenium and (no. of participants)	Country/population	Dose and (form) of Se required to reach maximal expression of selenoprotein P	Plasma selenium range associated with maximal selenoprotein P	(Reference) & comments
RCT, 16 weeks intervention with 200, 400 or 700 μg/day selenite, selenomethionine or Se-enriched yeast ($n = 81$)	US, men & women ≥18 years	>100 μg/day(US habitual diet)	≤122 ± 13 ng/ml	(Burk et al., 2006)Sepp1 maximal at baseline selenium intake of >100 μg/day (US habitual diet) and plasma selenium of 122 ± 13 ng/ml
1 year supplementation trial with 50, 100 or 200 μg/day selenomethionine($n = 261$)	US, population aged >18 years, men and women	≤109.1 ± 43.6 μg/day(US habitual diet)	≤142 ± 23.5 ng/ml.	(Combs et al., 2012). Sepp1 was maximal at study baseline, therefore Sepp1 may be maximal at intake/status equal to or below baseline data
RCT, 20 week intervention with 10, 20, 30, 40 μg/day selenomethionine ($n = 52$)	New Zealand, population aged 19–59, men and women	>69 μg/day(selenomethionine)	>88 ng/ml	(Duffield et al., 1999). Relatively small doses of Se supplement given in a low Se intake population, maximum level of Sepp1 not likely reached (based on US data) therefore values quoted as >highest intake/status investigated.
RCT, double blind placebo controlled 10 week supplementation with 50, 100 or 200 μg/day Se-enriched yeast or 50 μg/day Se-enriched onion meals ($n =$ 120)	UK, population aged 50–64 years, men and women	105 μg/day(habitual diet & Se-enriched yeast/Se-enriched onion meals (γ glutamyl Se-methylselenocysteine in onions)	125 ng/ml	(Hurst et al., 2010) Sepp1 maximal after 50 μg/day supplemental selenium as Se-enriched yeast or se-enriched food (onions), in additional to habitual intake ~55 μg/day. Sepp1 responded to different dietary relevant forms of Se.
11 week intervention with 200 μg/day Se-rich yeast, selenate or Se-rich wheat ($n = 50$)	Finland, men aged 36–60 years with low plasma selenium (<77 ng/ml)	>60–<200 μg/day(selenate, Se-rich yeast and Se-rich wheat resulted in maximal Sepp1)	>90–<130 ng/ml	At 2–4 weeks when Sepp1 was maximal with all 3 Se forms (Persson-Moschos et al., 1998) plasma Se was 120–130 ng/ml in Se-rich wheat and yeast groups (Levander et al., 1983) and ~90 ng/ml in selenate groups. Habitual intake was 62 μg/day at study baseline—Sepp1 not maximal in placebo group with ~50–60 μg/day Se intake
16 week intervention with 200 μg/day Se-rich yeast, selenate or selenate ($n =$ 45)	Finland, men, mean age 51 ± 7	~110 μg/day	≤110 ng/ml	Sepp1 maximal (Persson-Moschos et al., 1998) at baseline plasma selenium (110 ng/ml) (Alfthan et al., 1991) and baseline habitual intake ~110 μg/day (Alfthan et al., 1991)
RCT, 20 week intervention with selenomethione: 13, 24, 37, 48, 61 μg/day or selenite: 15, 31, 47, 52, 66 μg/day ($n = 120$)	China, men and women aged ≥18 years	>76 μg/day(selenite, selenomethionine)	>88 ng/ml	(Xia et al., 2005) Sepp1 not maximal at highest dose given 66 μg/day in addition to habitual intake
RCT, 40 week intervention with 21, 35, 55, 79, 102, 125 μg/day l-selenomethionine ($n = 98$)	China	49 μg/day(l-selenomethionine)	98 ± 15 ng/ml	(Xia et al., 2010). Habitual intake of 14 μg/day, maximum expression at 40 weeks after supplementation with 35 μg/day selenomethionine +14 μg/day habitual intake.

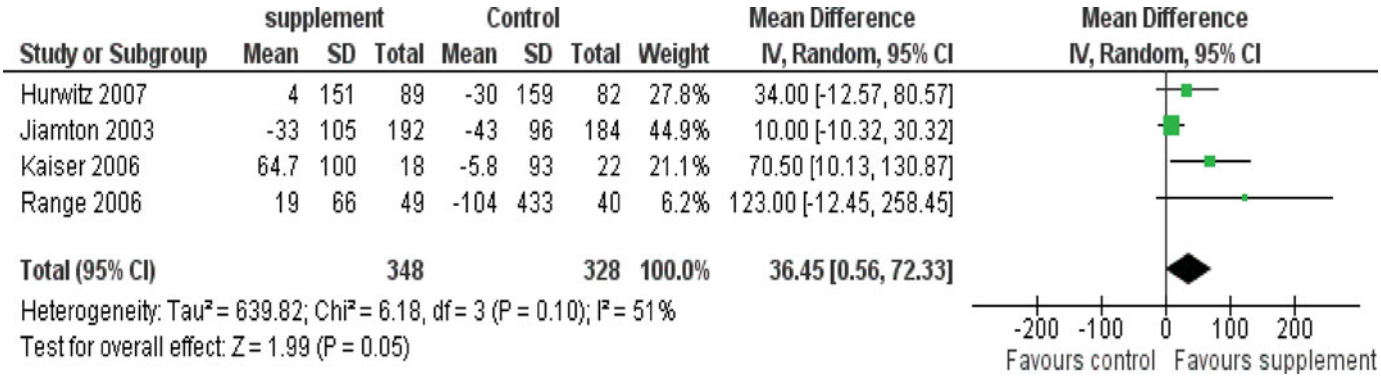

Figure 2 Forest plot of the effect of selenium supplementation on change in CD4 count from baseline. (color figure available online.)

($I^2 = 51\%$; Figure 2). Due to the limited number of studies included in this analysis (Jiamton et al., 2003; Kaiser et al., 2006; Range et al., 2006; Hurwitz et al., 2007), sensitivity or subgroup analysis to investigate the potential reasons for the heterogeneity was not possible.

Viral Load

Seven RCTs reported viral load data, including 1659 participants with between 18 and 238 participants per arm. There appeared to be a significant effect of selenium supplementation on log[viral load] levels post-intervention (WMD: −0.10; 95% CI: −0.19, 0.00; $p = 0.05$; 7 studies, 1659 participants; $I^2 = 0\%$; Figure 3). However to assess the effect of baseline status on viral load post selenium supplementation, viral load change data would be required. Unfortunately, these data were only presented by two studies and so further investigation is required.

Overall, there was high heterogeneity in the HIV disease state of the participants; for example, baseline CD4 and viral loads (indicative of HIV disease progression) were variable between studies and between treatment groups in the same studies at baseline. Selenium intervention may only be beneficial for certain disease states. However, due to there not being enough studies with defined populations in terms of disease progression, it is difficult to conclude further. In order to assess the effect of selenium in HIV, the populations studied must be clearly defined in terms of baseline disease status (CD4 count and viral load) to enable further meta-analysis.

Selenium and Immunity

Selenium plays a role in immune function (for reviews on potential mechanisms, including discussion of selenoproteins (Arthur et al., 2003; Fairweather-Tait et al., 2011) the work undertaken and presented as part of this EURRECA selenium review included assessment of selenium and role in immune function for the elderly population (>50 years). A total of 52 full text papers were screened, and six studies, comprising eight papers, were included in final analysis. Study characteristics and validity details were assessed (Collings et al., 2012). Of the included studies, three reported infectious episodes as the primary outcome, three reported antibody titers, four reported lymphocyte proliferation (including skin hypersensitivity tests), and one study reported severity of infection as an outcome. All studies included were RCTs of parallel design, and all were multivitamin or multivitamin/multimineral supplements. Intervention duration ranged from 8 weeks (Allsup et al., 2004) to 2 years

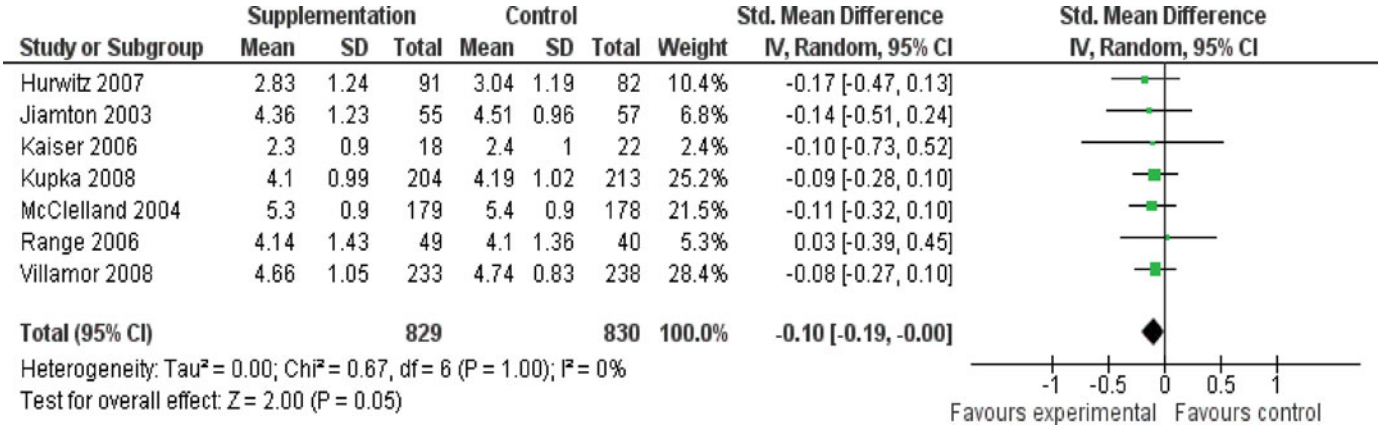

Figure 3 Forest plot of the effect of selenium supplementation on post-intervention log[viral load]. (color figure available online.)

(Girodon et al., 1997; Girodon et al., 1999). The selenium content of the supplements ranged from 10 μg (Bogden et al., 1994) to 100 μg (Galan et al., 1997; Girodon et al., 1999), and vitamin E content of the supplements ranged from 10 mg (Graat et al., 2002) to 80 mg (Liu et al., 2007) (all studies included vitamin E in the study design). The populations studied were a mixture of free-living and institutionalized elderly people (mixed gender), aged over 59 years.

No effect of intervention was reported for desired outcomes in four studies (Galan et al., 1997; Graat et al., 2002; Allsup et al., 2004; Liu et al., 2007), although Galan did report some changes to CD19, CD2, and IL-1. Bogden et al. (1994) reported increased response to recall antigens in the supplemented group (which comprised 10 μg selenium in the multimineral supplement), which was not observed in the placebo group. Chandra (1992) reported a lower likelihood of illness due to infection in the supplemented group compared to the placebo group. A higher percentage of participants with no reported infections was observed in the supplement group compared to the placebo group (Girodon et al., 1997; Girodon et al., 1999).

Overall, some improvements in immune status biomarkers were reported by the majority of studies (four out of the six included). However, all of the interventions included a number of other vitamins and minerals and interplay with these vitamins cannot be excluded. Thus, no clear conclusions on the effect of selenium on the immune function parameters investigated in the elderly population can be made.

Selenium and Fertility

Six studies met the inclusion criteria; all but one included study (Bleau et al., 1984) were RCTs (Scott et al., 1998; Hawkes and Turek, 2001; El-Bayoumy et al., 2002; Hawkes et al., 2009; Safarinejad and Safarinejad, 2009). Of the RCTs, four studies administered a selenium supplement and one was a feeding trial within a nutrition center (high selenium diet versus low selenium diet) (Hawkes and Turek, 2001). No studies were deemed to be of low risk of bias (Collings et al., 2012). One study did not report the method of randomization and only two reported a verification of the doses used. A total of five included studies presented data for sperm count. In summary, sperm count was not significantly affected by selenium intervention in the reported studies (p for overall effect 0.37) and heterogeneity was low (I^2 28%). Four included studies reported sperm motility and selenium appeared to have a significant influence on sperm motility. Only three studies reported sperm morphology as an outcome. Hawkes and Turek (2001) reported morphology as "% amorphous" rather than "% normal" and thus these results could not be included in a comparison. With only two studies with comparable data, meta-analysis could not be carried out. Safarinejad and Safarinejad (2009) reported a significant improvement in sperm morphology (reported as "strict morphology" expressed as % normal; increase in % normal from 22.1 ± 2.6 to 26.1 ± 2.9, $p = 0.03$), and Hawkes et al. (2009) reported an increase in % normal sperm morphology, but this was not significantly different in the Se-supplemented group compared to the placebo group.

Selenium and Total/Advanced Prostate Cancer Risk

A systematic review and meta-analysis to evaluate the association between selenium and prostate cancer risk was completed in collaboration with WCRF/AICR (Hurst et al., 2012). Nonlinear meta-analysis methods including fractional polynomials and cubic spline plots were used to investigate the relationship between plasma/serum/toenail selenium and total/advanced prostate cancer risk. The association between selenium and reduced prostate cancer risk was determined over relatively narrow ranges of plasma/serum selenium (120–170 ng/ml) and toenail selenium (0.85–0.94 μg/g). Further data are required from low selenium status populations to investigate potential "optimal" range of selenium status associated with reduced risk.

Multiple Micronutrients (Interactions)

Interactions or synergistic effects between selenium and vitamin E (Blot et al., 1993), iodine (reviewed in Arthur et al. (1999)), manganese, vitamin B12, copper (Salonen et al., 1991), or ascorbic acid (e.g., Martin et al., 1989) have been indicated but the requirement for selenium dose and form when multiple micronutrient deficiencies occur together or in certain combinations of deficiency/excess is not known. The influence of vitamin E was assessed were possible in the EURRECA systematic reviews on the effect of selenium intake/status on health outcomes (Collings et al., 2012), but data were lacking.

The interaction of selenium with other dietary components is a complex but important area for future research, especially for the interaction of selenium with iodine or vitamin E, highlighted below in "Research gaps and priorities" and also independently recommended in the iodine EURRECA micronutrient review summary document (Ristić-Medić et al., 2012).

Polymorphisms

Polymorphisms that have been indicated to be significantly associated with selenium biomarker status and health outcome(s) were identified using systematic search methodology and the results from relevant studies ($n = 22$ in total) are displayed in Table 3.

Polymorphisms associated with selenium status biomarkers. Several polymorphisms in selenoprotein genes including GPX1, SEP15, SEPP1, and GPX4 may be significantly associated with altered selenium status biomarkers (Table 3); however, the majority of data available to date are from relatively small studies with $n < 60$ per genotype investigated (Meplan et al., 2007; Meplan et al., 2008; Lei et al., 2009; Malling et al., 2009; Meplan et al., 2009; Cavar et al., 2010; Xiong et al., 2010) or up to $<n =$

Table 3 Significant associations between single nucleotide polymorphisms (SNPs)/genotype and selenium status biomarkers/health outcomes

Gene/genotype/SNP	Significant status biomarker interaction/association with SNP	Health outcome	Details of significant interaction with selenium status and/or health	Reference
GPX1				
GPx1 Pro198Leu	GPx activity; blood Se		Significant association between GPx1 activity and Se concentration in Pro/Pro and Pro/Leu genotypes	(Jablonska et al., 2009)
(rs1050450)	Whole blood Se; GPx activity		Whole blood Se and GPx1 activity in Pro/Leu and Leu/Leu groups were significantly lower compared to Pro/Pro group	(Lei et al., 2009) (Xiong et al., 2010)
	GPx activity		GPx1 activity was significantly lower in women with Pro/Leu or Leu/Leu GPX1 genotype compared with Pro/Pro genotype (similar difference not in males)	(Malling et al., 2009)
	Serum Se	Prostate cancer, (high-grade)	Decreased risk of prostate cancer and high-grade prostate cancer in subjects carrying T alleles for GPX1 SNP rs1050450 per 10μg/L increase in serum Se concentration	(Steinbrecher et al., 2010)
GPx1 -593 C/T	Serum Se GPx activity		T allele carriers (CT and TT) had higher pre-supplementation blood Se levels than CC homozygotes	(Cavar et al., 2010)
SEP15				
SEP15 (rs5879) (rs540049)	Plasma GPx activity and Se concentration		Decreased activity of GPx3 observed in patients with rare homozygote genotype (Sep15 rs5659 AA genotype/Sep15 rs540049 TT genotype)	(Steinbrecher et al., 2010)
Sep15 1125 G/A (3'UTR)	Plasma Se	Lung cancer	Significant association between Se concentration and lung cancer risk for GG, GA, AA genotypes	(Jablonska et al., 2008)
Sep15 gene (rs561104)	Plasma Se	Prostate cancer mortality	Significant interaction between rs561104 genotype and quartiles of Se for prostate cancer mortality (inverse association: G allele carriers over quartiles of Se status)	(Penney et al., 2010)
SEPP1				
SePP1 gene: rs3877899 (Ala234Thr);	GPx/SePP isoforms		Positive correlation between lymphocyte GPx4 and SePP 60 kDa isoform at baseline in GA individuals, but not GG.	(Meplan et al., 2009)
rs7579 (G/A position 25191 3'UTR)	Plasma Se GPx3 activity Lymphocyte GPx1 activity SePP concentration Erythrocyte TR1 concentration		Significant effect of SNP r25191g/a on plasma Se concentration post-supplementation- higher plasma Se concentrations in homozygotes of AA genotype Interaction between haplotype (presence of both r25191g/a and Ala234Thr SNPs in SePP) and BMI on plasma Se Individuals with GG genotype for r25191a/a had significantly increased GPx3 activity following Se supplementation compared to GA genotypes Increase in GPx1 activity post-Se supplementation was significant in females and males with GA and GG genotype for SNP Ala234Thr respectively. SNP r25191g/a affected SePP concentrations post-supplementation, with a significant genotype x gender interaction: GA males had higher plasma SePP Erythrocyte TR1 concentrations were lower in women with GA genotype compared with GG genotype (pre-and post-supplementation with Se)	(Meplan et al., 2007)

GPX4				
GPx4 C718T	GPx4 activity Lymphocyte GPx1 protein GPx3 activity		Difference in GPx4 activity between genotypes was significant at 2 and 4 weeks washout following Se supplementation, with lower activity in TT subjects TT females showed a rapid and significant drop in GPx1 post-supplementation (TT males no significant decrease) CC subjects had higher GPx3 activity than TT subjects post-supplementation	(Meplan et al., 2008)
OTHER				
SOD1 (rs10432782); SOD2 (rs2758330)	Plasma Se	Aggressive prostate cancer	Men with AG alleles for SOD2 rs2842958 SNP had lower levels of plasma Se than those with GG alleles Relative risk for aggressive prostate cancer in men with GG or GT for rs2758330 increased with increasing plasma Se levels (comparing men in highest Se quintile with lowest quintile)	(Abe et al., 2011)
SOD2 rs4880 (C/T position 47)	Plasma Se	Aggressive prostate cancer	Men with VV or VA allele (rs4880) and high plasma Se (>140–221 ng/ml) had increased risk of aggressive prostate cancer. AA genotype & high selenium levels had decreased risk.	(Chan et al., 2009)
MnSOD gene (Val/Ala codon 16)	Plasma Se	Aggressive prostate cancer	Significant increased risk of clinically aggressive prostate cancer for men with SOD2 AA genotype and plasma Se level in lowest quartile compared to men with VA/VV genotype	(Li et al., 2005)
GSTM1 null and non-null;	Plasma Se		Plasma Se level was inversely related to aflatoxin B1-albumin adducts: association significant in subjects who were GSTM1-null	(Chen et al., 2000)
GSTT1 null and non-null	Erythrocyte & plasma GPx		GSTM3*A/*A genotype - significantly higher activity of RBC and plasma GPx than carriers of the GSTM3 *B allele (either *B/*B or *A/*B). GSTT1 null genotype carriers had significantly higher plasma GPx activity compared to GSTT1(+) carriers	(Reszka et al., 2007)
BRCA1 mutations	Plasma Se		BRCA1 carriers: after supplementation higher Se status and lower oxidative DNA damage than carriers who have not been supplemented with Se	(Dziaman et al., 2009)
	Toenail Se		BRCA1 mutation carriers only: toenail Se inversely related to chromosomal damage following exposure to gamma irradiation	(Kotsopoulos et al., 2010)
Leptin gene mutation	Plasma Se, GPx and erythrocyte GPx		Plasma Se, GPx and erythrocyte GPx significantly lower in patients with leptin gene mutation compared to controls with no mutation	(Ozata et al., 2000)
HbSS and HbAA haplotypes	GPx activity	Sickle cell disease	Sickle cell anemia (HbSS) associated with significantly lower mean Se-GPx activity compared with HbAA controls	(Ren et al., 2008)
SBP2 gene mutations	GPx3 and serum Se		Baseline GPx3 activity and serum Se concentrations were significantly lower in SBP2 deficient (homozygous for SBP2 Q540R mutation) compared to heterozygous non-affected siblings	(Schomburg et al., 2009)
Metallothionein (MT1A +1245A/G)	GPx activity		Participants with CG haplotype for MT1A had higher GPx activity compared to CG haplotype.	(Giacconi et al., 2010)

200 with particular genotype (Jablonska et al., 2009) and so the SNPs require further investigation with respect to relevance at a population level. Several independent studies are in agreement for a significant association of GPX1Pro198Leu SNP with GPx activity, such that certain groups with Pro/Leu and Leu/Leu GPX1 genotypes were associated with significantly lower GPx activity in blood compared with those with Pro/Pro GPX1 genotype (Lei et al., 2009; Malling et al., 2009; Xiong et al., 2010). Other inter-individual variations have also been associated with a significant difference in plasma or erythrocyte GPx activity including certain GSTM1, GSTT1, MT1A genotypes, SBP2 or leptin gene mutations (Table 3) (Ozata et al., 2000; Reszka et al., 2007; Schomburg et al., 2009; Giacconi et al., 2010).

Polymorphisms associated with selenium status and health outcomes. SNPs that have been identified to have a significant association with both a selenium status biomarker and health outcome are presented in Table 3. For SNPs in selenoprotein genes associated with health outcomes see reviews (Rayman, 2009; Fairweather-Tait et al., 2011; Hesketh and Meplan, 2011). Certain inter-individual variations in SOD2 have been significantly associated with aggressive prostate cancer risk and low plasma selenium status (Li et al., 2005; Chan et al., 2009; Abe et al., 2011), detailed in Table 3. Also, men carrying T alleles for GPX1 SNP (rs1050450) or G allele for SEP15 SNP (rs561104) have significant changes in high-grade prostate cancer risk or prostate cancer mortality respectively over a range of plasma/serum selenium concentration (Penney et al., 2010; Steinbrecher et al., 2010). A significant association between SEP15 1125 G/A (3'UTR), plasma selenium and lung cancer risk was also reported (Jablonska et al., 2008) (Table 3). Published data suggest that SNPs in SEPP1 are linked with prostate cancer risk (Cooper et al., 2008; Steinbrecher et al., 2010) but the association of SEPP1 SNPs with cancer risk over a range of plasma selenium or selenoprotein P concentrations is not known. With the growing evidence for associations between SNPs and micronutrient status/health, polymorphisms may be important factors to consider when evaluating the data/evidence base for selenium, especially for selenium requirements in certain disease states, e.g., men who already have prostate cancer, have low selenium status, and known SNP linked with selenium status and aggressive cancer risk.

The database (http://www.php06.tno.nl/eurreca) on polymorphisms in relation to micronutrient status and health constructed as part of the EURRECA network also includes relevant data on zinc, iron and B12 (Doets et al., 2013; Harvey et al., 2013; Lowe et al., 2013). The database allows easy upload for newly reported relationships and search options to retrieve all SNPs and health outcomes related to selenium. Inclusion of new data from relevant publications and maintenance of the database will be taken care of by the Micronutrients Genomic Project (http://www.micronutrientgenomics.org). The database will be extended with possibilities to include expert reports on the reliability of the relationships between polymorphisms and micronutrient status and health.

INTEGRATING THE EVIDENCE

Assessment of dose response data is more appropriate for selenium (rather than factorial estimates) due to the potential beneficial effect over a relatively narrow range of intake/status. The intake of selenium required to maximize plasma glutathione peroxidase (GPx3) has been used in the past to determine requirements. However there is a wealth of new evidence published to suggest that the intake required to maximize the predominant selenoprotein in blood, plasma selenoprotein P, may be more appropriate. There are several high-quality dose response randomized controlled trials published within the last few years that may be used for meta-analysis of the intake-status relationship for plasma selenoprotein P.

Currently using the data on the effects and impact of genotype or individual variability on selenium requirement at a population level it is not possible to draw conclusions as results from large scale studies are not available. The polymorphism data shows that GPx activity in blood, used as a marker for dietary reference value calculations is prone to fluctuations due to other factors not linked to intake, including inter-individual variation, SNPs and health status.

A biological network was built based on the biological interactions of selenium (http://wikipathways.org/index.php/Pathway:WP15). In this network, interactions with other micronutrients such as manganese, copper, vitamin C, vitamin E, iodine, calcium, zinc, vitamin B6, vitamin B12, folate, and iron were included. The network biology model for selenium and the "health space" concept compiled as part of the EURRECA research activities (van Ommen et al., 2008; Bouwman et al., 2012) could aid the selection process regarding selenium related health status parameters that may be useful for dietary recommendations. In addition, these methods will help to integrate the selenium evidence with other micronutrients. The axis of the health space can be based on the network information and will include biological processes that are influenced by several micronutrients. The health space method will help basing dietary recommendations on biological requirements (activity of biological processes) and may inform regarding food based dietary recommendations, e.g., including selenium and other antioxidant micronutrients.

RESEARCH GAPS AND HARMONIES

The following research requirements and future research focus areas were discussed during the EURRECA expert consultation workshop in Leiden, Netherlands (EURRECA Network and Expert working group, 2011):

- research requirement for identification and validation of new selenium biomarkers over the range of health outcome effects, in particular over 70–200 μg/day. The effect of selenium intake on functional markers in target tissues should be defined. Metabolomics, genomics, and proteomics

approaches may be useful for functional biomarker identification, in both easily accessible blood fractions and target tissue.

- Regarding plasma selenoprotein P as a sensitive and reliable biomarker of selenium status, further research should be completed to ascertain the dose response effects of plasma selenoprotein P to a range of different selenium intakes and selenium forms (in particular in infants and elderly populations). The existence of selenoprotein P isoforms should be taken into account and association of selenoprotein P with health, including prostate cancer progression.
- The effect of single nucleotide polymorphisms (SNPs) on selenium status/health should be investigated further. Initial evidence shows that certain selenoprotein SNPs may be associated with significant effects on selenium status biomarker levels and may be linked with selenium status and disease risk. It is important to assess the combined effects of genotype and selenium status on health outcomes.
- Knowledge gaps regarding population groups and scaling: the selenium requirement for adolescent males should be considered carefully due to demand required for testes and prostate function during puberty. For intake-status-health data and optimal health outcomes, the most appropriate scaling options need to be elucidated to determine the requirement for children. This should include consideration of risk-benefit analysis and assessment of selenium biomarker levels in young population, where current data are limited.
- Future research is required on multiple micronutrient interactions and, in particular, the effect of iodine, vitamin E, and ascorbic acid intake on selenium metabolism, absorption, and status biomarkers.

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ABBREVIATIONS

CASP = Critical Appraisal Skills Programme
CD2, CD4, CD19 = Cluster of differentiation 2, 4 and 19
CEE = Central and Eastern Europe
EAR = Estimated average requirement
EC = European Commission
EURRECA = EURopean micronutrient RECommendations Aligned Network of Excellence
FFQ = Food frequency questionnaire
GPx = Glutathione peroxidase
GPx1 = Cellular glutathione peroxidase
GPx3 = Plasma glutathione peroxidase
GPX4 = Glutathione peroxidase 4 gene
GSTM1 = Glutathione S-transferase Mu 1 gene
GSTT1 = Glutathione S-transferase theta 1 gene
HIV = Human immunodeficiency virus
IL-1 = Interleukin-1
MMSE = Mini mental state examination
MT1A = Metallothionein 1A gene
NuGOwiki = The European Nutrigenomics Organisation webpages link
RCT = Randomized controlled trial
SBP2 = Selenocysteine insertion sequence (SECIS)-binding protein 2 gene
Sepp1 = Selenoprotein P
SEPP1 = Selenoprotein P gene
SEP15 = 15 kDa selenoprotein gene
SelH, SelI SelK = Selenoproteins H, I and K
SES = Socioeconomic status
SNP = Single nucleotide polymorphism
TrxR1-3 = Thioredoxin reductases 1-3
WCRF/AICR = World Cancer Research Fund/American Institute for Cancer Research
WHO = World Health Organisation
WMD = Weighted mean difference

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