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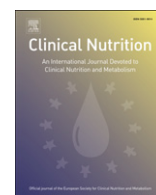
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## Review

# Micronutrients in pregnancy: Current knowledge and unresolved questions

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## SUMMARY

Micronutrient status is increasingly recognized to play an important role in the health and well-being of pregnant women and in the development and long-term health of the offspring. On 26th – 28th February 2009, The Child Health Foundation invited leading experts in this area to a scientific workshop at Obergurgl, Austria to review and critically discuss current knowledge, to identify issues that may need to be addressed in future recommendations, and to highlight priorities and opportunities for future research. This report summarizes updated key conclusions of the workshop with regards to micronutrients' intake and physiological role related to mother, placenta and fetus, as well as relevance for adverse pregnancy and long-term outcomes.

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## 1. Introduction

A balanced diet with adequate energy intake usually provides an adequate supply of the essential micronutrients. Although a balanced diet is generally accessible for the European population, specific groups have inadequate vitamin and mineral intakes, especially with regards to iron, iodide, folic acid, vitamin D and vitamin B<sub>12</sub>.<sup>1</sup> Micronutrient malnutrition represents an important challenge for public health worldwide, particularly in vulnerable population groups such as pregnant women. For example, a study in Hackney, London, showed that 78% mothers had an inadequate diet, meeting fewer than four of sixteen dietary reference intake values.<sup>2</sup> Upon follow-up at 9 months post-partum, over half of the un-supplemented, inadequate-diet group remained severely deficient in folate and had low serum ferritin levels. The adequacy of micronutrient intake during pregnancy is related to environmental, cultural and demographic variables, such as maternal age, clothing, geography or socioeconomic status (SES). Studies of plasma biomarkers suggest that SES significantly affects vitamin intakes. In

the European Prospective Investigation of Cancer Norfolk study, individuals in manual social classes, or those who lived in the most deprived areas had significantly lower levels of plasma ascorbic acid compared to those in non-manual social classes, or who lived in less deprived areas.<sup>3</sup> Similarly, in a study conducted in the United Kingdom, plasma concentrations of vitamins C and B<sub>12</sub>, riboflavin, and beta-carotene were lower in the low-SES group than in the high-SES group.<sup>4</sup> The French EVA study showed a positive association between SES participants and their selenium and carotenoid status.<sup>5</sup> Low income pregnant or breastfeeding women are at greater risk of insufficient vitamin and mineral intakes, inducing iron and vitamin A deficiencies.<sup>5</sup> Indeed, risk for pregnancy related diseases, malformations, preterm birth or low-birthweight (LBW) increases with socioeconomic deprivation and may therefore, at least in part, be related to poor micronutrient status.<sup>5</sup>

## 2. The physiological role of micronutrients in pregnancy

During the childbearing period, many women have inadequate dietary intake of several water soluble vitamins, especially folate. Micronutrient deficiencies or suboptimal/inadequate intakes may be associated with significantly elevated reproductive risks, ranging from infertility to fetal structural defects and long-term

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diseases.<sup>6,7</sup> Similarly, there is evidence demonstrating a role for micronutrient supplementation reducing the risk of some pregnancy disorders.<sup>8,9</sup> Micronutrients may affect pregnancy outcomes through alterations in maternal and fetal metabolism owing to their role/involvement in enzymes activity, signal transduction and transcription pathways, biological functions and oxidative stress, but the biological mechanisms underpinning these associations are not completely understood.<sup>10</sup>

## 2.1. Folic acid and vitamin B<sub>12</sub>

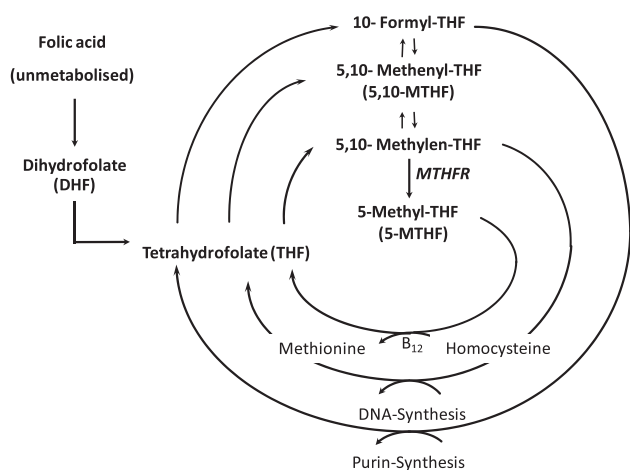
Folate and folic acid serve as the precursors for the coenzyme tetrahydrofolate in single-carbon transfer in the metabolism of amino acids and nucleic acids as well as substrate donors in the re-methylation of homocysteine into methionine, catalyzed by methionine synthase and 5,10-methylenetetrahydrofolate reductase (MTHFR). An inadequate dietary folate intake results in a reduction of DNA biosynthesis and thereby of cell division, leading to anemia, leucopenia, thrombocytopenia and other adverse effects.<sup>11</sup>

An overview of one-carbon metabolism is shown in Fig. 1. The predominant folate transport and storage form within the body is 5-methyltetrahydrofolate (5-MTHF). Synthetic folic acid is a precursor to the effective vitamin form. Folic acid is partly transformed in the mucosa cell to dihydrofolate (DHF) and tetrahydrofolate (THF) and later in the liver to 10-formyl-THF and 5,10-methylene-THF. MTHFR catalyzes the transformation of 5,10-methylene-THF into 5-MTHF.<sup>13</sup> A decrease in the methylation cycle is expressed in elevation of plasma total homocysteine (tHcy), or hyperhomocysteinemia (HHCY). HHCY can be inherited, i.e. genetic polymorphisms of MTHFR or acquired, i.e. folate and/or vitamin B<sub>6</sub>/B<sub>12</sub> deficiencies due to deregulation of their normal metabolism and/or low dietary intake.<sup>14–18</sup> As an example, homozygous genotypes with the C677T-MTHFR-polymorphism are 75% less active, when a point mutation at nucleotide 677 causes the replacement of cytosine with thymine. Insufficient 5-MTHF increases homocysteine because of inadequate re-methylation of homocysteine into methionine.<sup>19</sup> Indeed, Hcy, which is neurotoxic, is metabolized by re-methylation by homocysteine methyl transferase, requiring 5-MTHF and vitamin B<sub>12</sub>. Another Hcy elimination

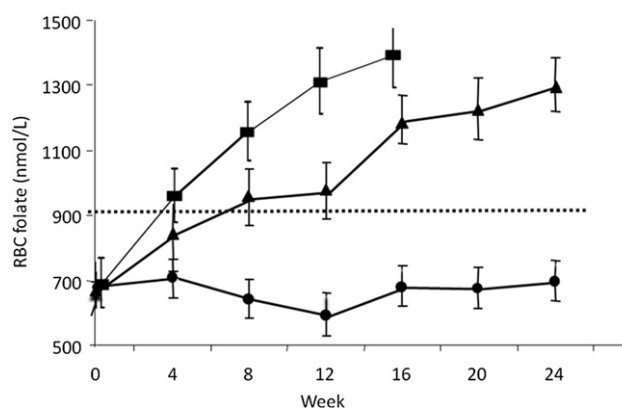
route is the trans-sulfuration pathway, where the conversion from homocysteine to cysteine takes place in two enzymatic steps, each of which involves vitamin B<sub>6</sub> as a coenzyme.<sup>20</sup>

### 2.1.1. Folic acid intake during pregnancy

Folate needs increase substantially in pregnancy owing to the enlargement of the uterus, the development of the placenta, and the increasing red cell volume of the mother, as well as the growth of the developing fetus. There is consistent scientific evidence that folic acid is of critical importance both pre- and periconceptionally in protecting the fetus from neural tube defects (NTDs).<sup>21</sup> Estimated folate requirements increase by 50% to 600 µg/day during pregnancy, and even if the daily diet consists of food rich in folates, the high requirement usually cannot be met through the consumption of unfortified foods alone. For this reason, internationally, periconceptional supplementation of 400 µg/day of folic acid is recommended for prevention of NTDs. Periconceptional supplementation of 800 µg/day of folic acid combined with multivitamins achieved a near 100% reduction of NTDs as well as marked reductions of congenital heart defects.<sup>22–24</sup> Daly et al.<sup>25</sup> found that red cell folate levels higher than 906 nmol/L are associated with maximal risk reduction of NTDs. In 1998 the German Nutritional Survey evaluated folate concentrations in serum and in red cells of women of childbearing age. Only 13% of the women showed a red cell folate level higher than 906 nmol/L.<sup>26</sup> With a daily dosage of 800 µg folic acid, the preventive red blood cell level of 906 nmol/L is reached within an average 4 weeks after the start of supplementation<sup>27</sup> (Fig. 2). In contrast, the current recommendation to supplement 400 µg folic acid daily starting at least 4 weeks before conception is not enough to achieve optimal red cell folate levels within 4 weeks; this requires at least 8–12 weeks of daily intake.<sup>28</sup> Though there might be an adaptation to increase absorption of nutrients during pregnancy, optimal folate levels have to be achieved around conception as the neural tube closes between 23rd and 27th day of pregnancy. Thus, if one wishes to maintain the current recommended supplementation period of 4 weeks before pregnancy, it may be preferable to increase the folic acid dosage to 800 µg to achieve a maximal preventive effect associated with a red cell folate level of greater than about 900 nmol/L. Maternal folic acid intake also correlates with birth-weight,<sup>29</sup> which may be explained through the role of folate in nucleic acid synthesis. Moreover, associations between maternal folate status and pathological changes in the placenta have been



**Fig. 1.** Overview of one-carbon metabolism. Modified from K. Pietrzik, I. Golly, D. Loew, Handbuch Vitamine, Elsevier Urban & Fischer 2008.<sup>12</sup> Folic acid is partly transformed in the mucosa cell to dihydrofolate (DHF) and tetrahydrofolate (THF) and later in the liver to 10-formyl-THF and 5,10-methylene-THF. Methylenetetrahydrofolate reductase (MTHFR) catalyzes the transformation of 5,10-methylene-THF into 5-MTHF.<sup>13</sup> Vitamin B<sub>12</sub> is essential for the conversion of 5-MTHF to 5,10-methylene-THF and, consequently, for the methylation cycle and the biosynthesis of DNA and RNA.



**Fig. 2.** Change in red blood cell (RBC) folate concentration (nmol/L) of childbearing women after a dosage of 800 µg (■) and of 400 µg (▲) folic acid per day over a long period of time. Placebo (●) was also considered. Blotted line indicates the lower RBC folate level (906 nmol/L) required as an optimum risk reduction of neural tube defects (NTDs). Modified from K. Pietrzik, I. Golly, D. Loew, Handbuch Vitamine, Elsevier Urban & Fischer 2008.<sup>12</sup>

reported (abruption placenta, infarct of the placenta), as well as with spontaneous abortion. These associations are also evident for homocysteine levels.<sup>30–33</sup> Interestingly, an increased risk of NTDs was associated with a high prevalence of a genetic variant in the pathway of homocysteine metabolism causing HHCY.<sup>13,34</sup> Results from this study showed that the incidence of NTDs not only correlates with the supply of folate but also that of vitamin B<sub>12</sub>. Women with a low folate supply combined with low blood vitamin B<sub>12</sub> levels have a drastically increased risk of NTDs. Vitamin B<sub>12</sub> is involved in homocysteine metabolism being a coenzyme of the methionine synthase and acting as a 5-MTHF methyl group donor from methylcobalamine to homocysteine. Biochemically, vitamin B<sub>12</sub> and 5-MTHF are closely linked in regard to homocysteine remethylation. Thus, an increased tHcy level could be a causal factor for NTD occurrence. These findings raise the potential that the most effective periconceptional prophylaxis to prevent NTDs could be the provision of both folic acid and vitamin B<sub>12</sub>. A meta-analysis of randomized trials demonstrated that daily supplementation with both 0.5–5.0 mg folic acid and 0.5 mg vitamin B<sub>12</sub> can reduce blood homocysteine concentrations by about a quarter to a third.<sup>35</sup> In particular, a folic acid dose of 800 µg was shown to have optimal dosage with respect to lowering Hcy levels.<sup>36</sup> It is possible that the biological potential of folate might be even higher by using 5-MTHF supplements, since significantly higher red blood cell folate levels were achieved by 5-MTHF in the form of calcium-L-methylfolate compared to folic acid supplementation.<sup>28</sup> Given that not only an increased risk for NTDs<sup>37</sup> but also various complications during pregnancy such as abruptio placenta, placenta infarction and spontaneous abortions are associated with MTHFR-polymorphism,<sup>38–40</sup> provision of 5-MTHF might optimize the benefits of the folate supply.

### 2.1.2. Folic acid and public health measures

Even though the importance of periconceptional folic acid supplementation for prevention of NTDs has been widely acknowledged and supported by both scientific and governmental recommendations throughout the world, its implementation is less than satisfactory. Folate supplementation should be recommended for all women who might become pregnant, and public health measures should be taken to ensure that the diet of all women who may bear children is enriched with an adequate amount of folic acid. The current recommendation to supplement 400 µg of folic acid at least 4 weeks before conception and in the first trimester of pregnancy should be re-assessed, particularly in areas where a general food fortification policy has not yet been implemented. Currently, more than 50 countries worldwide have national regulations for mandatory wheat-flour fortification with folic acid. In response to fortification there was a 26% risk decline of NTDs in the US and approximately 46% in Canada with higher reductions in high risk provinces (e.g. –78% in Newfoundland). Data from other countries support the benefit of those regulations. However, no European country has yet developed national regulations for mandatory folic acid fortification. The discussion on fortification remains controversial because of concerns regarding potential risks of chronic exposure to high-dose folic acid.<sup>41</sup> The safety of very high intakes of folic acid is really unknown, so the Institute of Medicine recommends a tolerable upper intake level for folic acid of 1 mg/day for adults.<sup>42</sup> Specifically, high doses of folic acid are suggested to mask anemia caused by vitamin B<sub>12</sub> deficiency thus leading to delayed diagnosis of neurological symptoms.<sup>43,44</sup> Moreover, the issue of folate and risk of cancer has received much public health attention as a result of a potential dual role of folic acid in carcinogenesis. It seems that folic acid supplementation prevents the development of tumors in normal tissues, whereas it accelerates the development and progression of already growing neoplasms.<sup>41</sup>

The timing and the dose of folate intervention appears to be responsible for this dual modulatory role of folate in carcinogenesis. However, evidence from randomized trials is lacking and inconclusive, consequently further investigations are needed in this field.<sup>45</sup> Similar discrepancies have been reported regarding the adverse effects of folic acid on zinc absorption, as some authors observed a significantly decreased zinc absorption when pregnant women received 350 µg/day iron-folic acid supplement for 2 weeks.<sup>46</sup> Nevertheless, a 12-wk placebo-controlled trial failed to detect any adverse effect of 400 µg/day folic acid supplementation on zinc absorption.<sup>47</sup>

## 2.2. Antioxidants

Reactive oxygen species (ROS), reactive nitrogen species and reactive chlorine species are synthesized in humans and animals under physiological and pathological conditions.<sup>48</sup> ROS oxidize biomolecules such as DNA, lipids, proteins, leading to cell injury and death. ROS are involved in female reproduction, in such physiological processes as the endometrial cycle, luteolysis, implantation, embryogenesis, and in pregnancy.<sup>49,50</sup> An imbalance in the equilibrium of pro-oxidants and antioxidants can result in oxidative damage (OS), a key element in the pathogenesis of several diseases.<sup>51</sup> Antioxidants maintain homeostasis *via* effects on redox status and/or redox-sensitive signaling pathways and gene expression.<sup>52</sup> By contributing directly and indirectly to antioxidant defense, antioxidants enhance many aspects of the immune response and limit pathological aspects of the cytokine-mediated response.<sup>48</sup> Many antioxidants are derived from the diet, such as vitamin E and other tocopherols, vitamin C, β-carotene, whereas proteins and peptides, such as glutathione (GSH), ceruloplasmin, and metallothionein are synthesized endogenously.<sup>53,51</sup> Other micronutrients such as magnesium, copper, zinc, manganese and selenium are involved in antioxidant defense as cofactors of enzymes (i.e., copper–selenium–zinc in superoxide dismutase (SOD), selenium in GSH peroxidase).<sup>48</sup>

The demands of normal pregnancy, especially during the third trimester and at parturition, impose considerable systemic oxidative, metabolic and inflammatory stress.<sup>54</sup>

### 2.2.1. Oxidative and inflammatory stress and the onset of preeclampsia

Preeclampsia remains a frequent and potentially dangerous complication of pregnancy, affecting 2–5% of pregnant women worldwide, and it is a major cause of maternal death.<sup>55</sup> As delivery is the only known cure, and because it can occur from 20 weeks of gestation onwards, preeclampsia can also lead to preterm delivery, contributing to high neonatal mortality and morbidity. Thus, preeclampsia is associated with both a maternal and a fetal syndrome. The maternal syndrome of preeclampsia has previously been ascribed to generalized maternal endothelial cell dysfunction. However, the endothelial dysfunction is part of a more generalized intravascular inflammatory reaction involving intravascular leukocytes as well as the clotting and complement systems.<sup>54</sup> In preeclampsia both systemic oxidative stress and inflammatory state are significantly enhanced as part of the maternal syndrome. The condition evolves in two stages (Fig. 3): stage 1 occurs without symptoms in the first half of pregnancy, due to poor placentation. This causes an abnormally high pressure and pulsatile uteroplacental blood that induces oxidative stress and increased synthesis and release of pro-inflammatory factors. During placentation, trophoblast-cell invasion into the placental bed is inadequate, with deficient remodeling to enlarge the spiral arteries.<sup>56</sup> The disturbed pattern of blood flow leads to reduced growth of the branches of the placental villous tree, namely the narrow muscular arteries



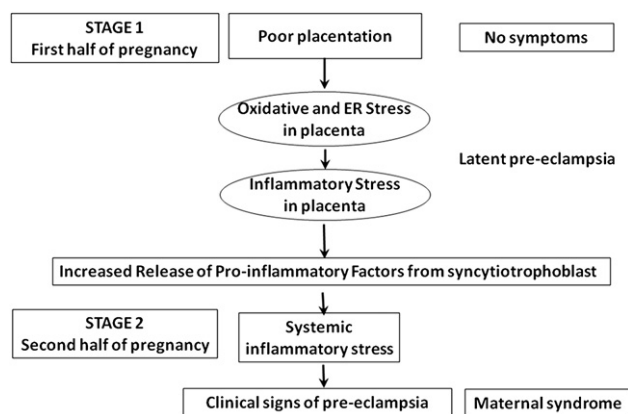


Fig. 3. Stages and factors involved in preeclampsia onset and development. ER: Endoplasmic reticulum.

which perfuse lobules intermittently. Numerous studies suggest that the “preeclamptic” oxidative stress arises from hypoxia/re-perfusion.<sup>57</sup> These include the demonstration of increased activity of enzymatic pathways leading to superoxide generation, direct measurement of free radical synthesis, activation of downstream signaling pathways and evidence of enhanced lipid peroxidation. Increased expression of stress response genes including heme oxygenase 1 provides further support to this hypothesis. The placenta appears to be the principal source of free radical synthesis but maternal leukocytes and the maternal endothelium are also likely contributors. Recent reports have suggested an important role for placental trophoblast NAD(P)H oxidase in free radical generation in preeclampsia.<sup>55,58</sup> Stage 2 preeclampsia presents at any time in the second half of pregnancy with the typical clinical signs, and is characterized by systemic inflammatory stress. Preeclampsia may be associated with fetal growth restriction, because the damaged placenta has a reduced functional capacity.

Maternal health status and dietary factors may affect the occurrence of preeclampsia. Chronic arterial and metabolic diseases such as hypertension, obesity, type 2 diabetes and metabolic syndrome are risk factors for preeclampsia. However, the mechanisms by which obesity may cause gestational diabetes and hypertensive disorders of pregnancy is still under investigation.<sup>59</sup> Several possibilities include the adverse effects of insulin resistance, elevated cholesterol and elevated leptin levels on blood pressure. Moreover, in pregnancy the placenta is the primary

source of leptin.<sup>60</sup> Remarkable increases in fetal and placental leptin levels have been associated with preeclampsia. Leptin and immune function are linked,<sup>61</sup> and in particular leptin has a pro-inflammatory role. Adipose tissue is a source of several other inflammatory factors besides leptin (for example interleukins-1, -6 and tumor necrosis factor alpha), complement factors and components of the coagulation/fibrinolytic cascade. IL-6 stimulates C-reactive protein synthesis which is positively correlated with obesity, insulin resistance, elevated TNF- $\alpha$  and endothelial dysfunction.<sup>62</sup> Low grade systemic inflammatory activation is typical of preeclampsia but the extent to which leptin, presumably of placental origin, contributes is still not known. Diet may also be relevant. Both excessive eating and consuming a poor quality diet are associated with more systemic inflammatory stress. In the non-pregnant state, meals and especially fat rich meals cause transient inflammatory stress<sup>63,64</sup> which may be sustained if the intervals between meals are short.

### 2.2.2. Role of antioxidants in preeclampsia

Some nutrients may protect against preeclampsia. Calcium supplementation has been reported to reduce the incidence of preeclampsia, particularly in geographic regions with low calcium intakes.<sup>65</sup> Although the mechanism is obscure, supplements may be assumed to increase chylomicron clearance after meals<sup>66</sup> which could reduce postprandial inflammatory stress.

Omega-3 long chain PUFA may also attenuate inflammatory responses. However, the evidence from human studies is inconsistent if not conflicting.<sup>67</sup> There is no conclusive evidence that supplementation of omega-3 long chain PUFA would be beneficial for preventing preeclampsia<sup>68</sup> and some data even suggest a risk increasing effect.<sup>69</sup>

The antioxidant vitamin E is known to have multiple actions in addition to preventing lipid peroxidation, including inhibition of NAD(P)H oxidase activation and the inflammatory response.<sup>70</sup> Given the abnormally low plasma vitamin C concentrations reported in preeclampsia, a combination of vitamins C and E has been postulated as a prophylactic strategy for prevention of preeclampsia.<sup>71</sup> However, there is no evidence that high doses of vitamin C and vitamin E (1000 mg and 400 IU, respectively) combined reduce the occurrence of preeclampsia.<sup>72</sup> In particular, some randomized controlled trials involving a large number of pregnant women have shown a lack of benefit in women receiving antioxidants,<sup>73–76</sup> as reported in Table 1. Therefore, the use of such high-dose antioxidants cannot be justified in pregnancy.

Table 1  
Characteristics and results from randomized controlled trials intervention studies indicating no effects on preeclampsia of high doses of vitamin C and Vitamin E (1000 mg and 400 IU, respectively) combined.

Study design	Result	Reference
VIP Trial Women identified as at increased risk of preeclampsia from 25 hospitals (UK). From the second trimester of pregnancy until delivery. Vitamin group = 1199; Placebo group = 1205.	Risk Ratio (95% CI) = 0.97 (0.80–1.17); $p = 0.754$	70
Australian Collaborative Trial of Supplements (ACTS) study Multicenter, randomized trial of nulliparous women between 14 and 22 weeks of gestation. Vitamin group = 935; Placebo group = 942.	Risk Ratio (95% CI) = 1.20 (0.82–1.75); $p = 0.57$	71
WHO multicentre randomized trial Pregnant women at high risk for preeclampsia in populations of low nutritional status from India, South Africa, Peru and Vietnam. Vitamin group = 687; Placebo group = 678	Risk Ratio (95% CI) = 1.0 (0.9–1.3)	72
Multicenter, randomized, double-blind trial involving nulliparous women at low risk for preeclampsia (USA). Vitamin group = 5088; Placebo group = 5066.	Risk Ratio <sup>a</sup> (95% CI) = 1.07 (0.91–1.25); $p = 0.42$	73

<sup>a</sup> Severe pregnancy-associated hypertension alone or severe or mild hypertension with elevated liver-enzyme levels, thrombocytopenia, elevated serum creatinine levels, eclamptic seizure, indicated preterm birth, fetal growth restriction, or perinatal death.

These trial results add to the broader literature in cardiovascular medicine where antioxidant supplements generally have no effect on the progression of cardiovascular disease, despite good evidence for an association of these disorders with biomarkers of oxidative stress. Whether these trials suggest that oxidative stress is not part of the pathogenesis of preeclampsia or the dose or the choice of antioxidant prophylaxis is inappropriate, is unknown. The susceptibility to oxidative stress of erythrocytes has been found to be increased in preeclampsia as early as 14–20 weeks gestation prior to onset of the clinical disease.<sup>77</sup> This depended on glucose-6-phosphate dehydrogenase (G6PD) activity, essential to maintain intracellular NADPH activity, which was lower in preeclamptic pregnancies compared to controls and unaffected by antioxidant treatment with vitamins C and E. These findings provide the first evidence that decreased G6PD activity in preeclampsia causes impaired redox regulation in erythrocytes and might explain why antioxidant vitamin C/E supplementation in the VIP trial did not protect from preeclampsia.<sup>77</sup>

Selenoenzymes play a critical role in regulating antioxidant status, through the selenoproteins such as GSH peroxidases (GPx). GPx removes the products of reactions with hydroperoxides and oxidized lipoproteins, limiting adverse effects on the endothelium. It is therefore of some interest that a poor maternal selenium (Se) status may be a predictor of preeclampsia, given that women who develop overt preeclampsia have a lower Se status.<sup>78</sup> Vanderlelie et al.<sup>79</sup> studied the tissue levels of endogenous antioxidant proteins (SOD, GSH peroxidase, thioredoxin reductase and thioredoxin) and of lipid and protein oxidation in placental samples from normal and preeclamptic pregnancies and found a decreased enzymatic antioxidant capacity in placental tissue from preeclamptic women compared to controls. Highly significant reductions in serum Se concentrations and plasma GSH peroxidase activity were found in pregnancy *per se* compared to non-pregnant subjects.<sup>80</sup> However, even lower levels were found in preeclamptic mothers and their babies. Subsequently, both preeclamptic mothers and their infants had significantly increased levels of oxidative stress-markers and reduced placental GSH peroxidase activity. In rats, low Se intake causes a preeclampsia-like syndrome<sup>81</sup> which is consistent with the human observations. Oxidative stress related to preeclampsia might be a consequence of reduced antioxidant defense pathways due to reduced Se availability. Reduced GPx may be related to increased generation of toxic lipid peroxides contributing to the endothelial dysfunction and hypertension of preeclampsia.

### 2.3. Vitamin A

Vitamin A is obtained from the diet either as pre-formed vitamin A in the form of retinol or retinyl-esters, or as provitamin A-carotenoids. The highest content of pre-formed vitamin A is found in liver and liver oils of marine animals. Yellow and green leafy vegetables provide significant amounts of provitamin A-carotenoids.<sup>82</sup> However, high doses (<6 mg/day) of provitamin A are needed to substitute pre-formed retinol.<sup>83</sup> Retinol is the endogenous metabolite and retinoic acid is the morphogenetically active compound. Vitamin A plays a key role in ocular retinoid metabolism and visual function as well as in cellular differentiation, related to embryonic development in particular lung maturation and immunity. Carotenoids exert antioxidant properties.

Fetal and neonatal vitamin A status depends on maternal vitamin A status. Fetal/neonatal synthesis of retinol binding protein is not sufficient to ensure continuous supply from liver stores. Hence, an adequate maternal vitamin A supply is essential to ensure normal fetal growth and development.<sup>11</sup> The Recommended Daily Allowance (RDA), that is the amount of vitamin A that should be taken to prevent vitamin deficiency, is about 0.8 mg/day.<sup>84</sup> A

large heterogeneity, however, exists with recommended intakes varying from 0.77 mg/day in UK<sup>85</sup> to 1.1 mg/day in D-A-CH countries.<sup>86</sup> Vitamin A deficiency during pregnancy seems to be associated with preterm birth, LBW and low neonatal liver stores.<sup>87–90</sup> Low vitamin A status of the newborn appears to contribute to the risk for bronchopulmonary dysplasia (chronic lung disease). Low neonatal liver stores and a low supply during lactation also appear to increase the risk for infection.<sup>90</sup>

The German Nutrition Society (DGE) recommends a 40% increase in vitamin A intake for pregnant women and a 90% increase for breastfeeding women. The American Academy of Pediatrics Committee on Nutrition (1998)<sup>91</sup> cites vitamin A as one of the most critical vitamins during pregnancy and the breastfeeding period, especially in terms of lung function and maturation. If the mother has suboptimal intake, her supply to fetus will be inadequate, as will later the vitamin A content of her milk. These inadequacies cannot be rectified by postnatal supplementation. A clinical study in pregnant women with short birth intervals or multiple births showed that almost 1/3 of the women showed plasma retinol levels below 1.4  $\mu\text{mol/L}$  which can be taken as borderline deficiency.<sup>92</sup> Despite the fact that vitamin A and beta-carotene rich food is generally available, risk groups for low vitamin A supply exist in the western world.

Even though 25% of the population have an inadequate retinol supply<sup>93</sup> pregnant women are routinely advised against consumption of liver, the major dietary source of pre-formed vitamin A. This advice is based on two main considerations. The first is that there is no doubt that synthetic retinoids, used for the treatment of severe acne are teratogenic.<sup>94</sup> Secondly there are case reports of teratogenicity associated with high-dose supplementation with, or overdose of, vitamin A. This raises the question which source of vitamin A supply in pregnancy and lactation can be considered adequate and safe. Alternative strategies to augment maternal vitamin A status are either selective supplementation with retinol, or supplementation/fortification with isolated  $\beta$ -carotene. However, the conversion of  $\beta$ -carotene to retinol may be limited and varies with genetic polymorphisms frequently found in the population.<sup>95</sup> Excessive dietary intake has been associated with teratogenicity in humans in 20 reports published until 1986, and no further reports since that time.<sup>94</sup> However, there is a lack of adequate data on a teratogenic effect of pre-formed vitamin A (retinol or retinylester).<sup>96</sup> In particular there are no data showing any relationship between consumption of liver and malformations. Nevertheless, a daily intake of more than 3 mg pre-formed Vitamin A (10,000 IU) should be avoided by women who are or who might become pregnant based on a precautionary principle.<sup>11</sup> During the first trimester of pregnancy vitamin A intake from liver or supplements is not recommended. However, especially during the third trimester, sufficient vitamin A intake that is 100–150% RDA is strongly recommended to ensure sufficient neonatal liver and lung stores of vitamin A for adequate tissue maturation.<sup>1</sup>

### 2.4. Vitamin D

Over the past decade observational studies in non-pregnant individuals have associated low 25-OH-vitamin D levels in plasma with an increased risk of various common chronic diseases such as colon, breast and prostate cancer, metabolic syndrome, hypertension, multiple sclerosis, type I diabetes, and inflammatory bowel disease, even though causality has not been firmly established.<sup>97</sup> In addition to its action on calcium metabolism and bone homeostasis, there are other physiologic systems in which vitamin D, the steroid hormone, 1,25-dihydroxyvitamin D (1,25[OH]2D), and its receptor generate biological responses.<sup>98</sup> For example, 1,25[OH]2D inhibits parathyroid hormone (PTH) secretion but promotes insulin

secretion, it inhibits adaptive immunity but promotes innate immunity. So vitamin D appears to exert anti-inflammatory and anti-microbial activity through the innate immune system and to inhibit cell proliferation while stimulating cell differentiation.<sup>99</sup>

Estimates of the prevalence of vitamin D deficiency or insufficiency vary greatly depending on the chosen cut-offs for serum 25-hydroxyvitamin D (25[OH]D) concentrations.<sup>100,101</sup> Indeed, the threshold for optimal 25[OH]D concentration could vary with regards to the expected effects, which may relate to bone health but also to the non-classical actions of vitamin D on maternal and fetal outcomes. In Australia and New Zealand currently a dietary vitamin D intake for pregnant women of 200 IU/day has been recommended<sup>102,103</sup> while 400 IU/day are recommended in the United Kingdom<sup>104</sup> and 600 IU/day in the United States and Canada.<sup>105</sup> Basing adequate intake allowances of vitamin D on vitamin D's actions on calcium and bone tissues is the subject of widespread controversy.<sup>106,107</sup> Given the evolving concept of vitamin D-sufficiency, it is currently considered that sufficiency may be defined by a serum 25[OH]D levels >75 nmol/L.<sup>100</sup> By this criterion, the prevalence of vitamin D insufficiency during pregnancy could be as high as 70% in western countries.<sup>108</sup> The related potential public health implications warrant attention.

Recent surveys confirm that 25[OH]D concentrations in pregnant women are not different from those of non-pregnant women and are significantly associated with ethnicity.<sup>109,110</sup> Vitamin D deficiency is more common in dark-skinned individuals living in northern countries where sunlight is less effective in producing vitamin D3 (1,25[OH]2D) in darker skin because the ultraviolet light is absorbed by the skin pigment. In addition, low sunlight exposure, the habit of covering the skin, and a diet low in vitamin D and calcium may all contribute to lower vitamin D concentrations in non-Western immigrants to northern countries.<sup>111,112</sup> A study in pregnant women of several ethnic backgrounds living in The Hague

(the Netherlands) showed that mean serum 25[OH]D concentrations were significantly lower in women of Turkish, Moroccan, and other non-Western groups than those in Western women.<sup>110</sup> A comparison of the observed serum 25[OH]D concentrations in the population of the USA measured in the NHANES III (1988–1994) and NHANES 2000–2004 studies suggests an overall decline in vitamin D status over the past 10–15 y.<sup>109</sup>

Vitamin D status may be critical with regards to pregnancy and fetal/infant outcomes.<sup>113</sup> Severe vitamin D deficiency causes modest hypocalcemia and secondary hyperparathyroidism and osteomalacia in non-pregnant adults, but no reports have documented worsening during pregnancy.<sup>114</sup> Despite the significantly higher needs of calcium to be passed on to the developing fetus, maternal adaptations provide the necessary calcium relatively independent of vitamin D status. Table 2 presents observational studies addressing the association between poor pregnancy outcomes and vitamin D.<sup>115–120</sup> Vitamin D deficiency seems to be associated with an increased risk of preeclampsia. The pathogenesis of preeclampsia involves several biological/molecular pathways that may be affected by vitamin D, including placental dysfunction/insufficiency, abnormal angiogenesis, excessive inflammation, and hypertension.<sup>121–124</sup> However, a recent study showed no association between vitamin D deficiency and preeclampsia<sup>125</sup> to suggest that low serum vitamin D levels in preeclampsia do not necessarily indicate dietary deficiencies but may be a consequence of placental dysfunction or a secondary consequence of systemic inflammation. The placenta synthesizes active 1,25[OH]2D from its precursor.<sup>125–127</sup>

A possible role of vitamin D in maintaining normal glucose homeostasis is discussed in the review by Peechakara et al.<sup>128</sup> In both animal and human studies, vitamin D depletion appears to be associated with insulin resistance and impaired insulin secretion.<sup>129</sup> In particular, a significant and strong association between

**Table 2**  
Studies examining the association between vitamin D status and diabetes or preeclampsia.

Outcome	Study Design	Result	Reference
Vitamin D deficiency and preeclampsia	Case-control study of pregnant women followed from less than 16 wk gestation to delivery. Nulliparous pregnant women who developed preeclampsia ( <i>n</i> = 55) or did not develop preeclampsia ( <i>n</i> = 219)	Adjusted serum 25[OH]D concentrations in early pregnancy 15% lower in women who subsequently developed preeclampsia compared with controls. Adjusted odds ratio (95% CI) = 2.4 (1.1–5.4)	115
	Longitudinal study included 170 healthy normotensive pregnant women. During the study, 160 women remained NT and 10 developed preeclampsia at the third gestational period. Thirty-two patients with preeclampsia and 20 normotensive women with singleton gestations in the third trimester.	Circulating levels of 1,25-[OH]2D not altered in women before they developed preeclampsia.	116
		Increased bone resorption and decreased bone formation occur in preeclampsia in both mother and fetus. Normal pregnancy: maternal coupling index (CI) of markers of bone turnover = +3.28; fetal CI = +11.8. Mild preeclampsia: maternal CI = +1.4; fetal CI = -30.85. Severe preeclampsia: maternal CI = -25.71; fetal CI = -70.02	117
Vitamin D deficiency and insulin resistance	Nested case-control study among a prospective cohort of 953 pregnant women (Omega Study). Incident GDM cases ( <i>n</i> = 57) and women who were not diagnosed with GDM ( <i>n</i> = 114)	Each 5 ng/ml decrease in 25[OH]D concentrations was related to a 1.29-fold increase in GDM risk. Odds ratio (95% CI) = 1.29 (1.05–1.6)	118
	Prospective study a convenience sample of 307 pregnant women.  Cross-sectional study was conducted on 741 pregnant women with no previous history of diabetes mellitus	Significant linear associations were therefore found between log-transformed 25[OH]D and fasting glucose, fasting insulin and HOMA-insulin resistance Prevalence of severe vitamin D deficiency in GDM patients higher than in normoglycaemic pregnancies. 25[OH] D levels: GDM pregnancies = (16.49 ± 10.44) vs normal pregnancy = (22.97 ± 18.25); <i>p</i> = 0.009. Strong correlation between the HOMA index and serum levels of vitamin D.	119  120

GDM: gestational diabetes mellitus.

vitamin D deficiency and  $\beta$ -cell dysfunction has been reported in 126 healthy, glucose-tolerant subjects.<sup>130</sup> Moreover, some studies have found a significant association between maternal plasma 25 [OH]D concentrations in early pregnancy and the risk for gestational diabetes mellitus (GDM), fasting glucose levels or the Homeostasis Model Assessment (HOMA) index<sup>118–120</sup> (Table 2).

#### 2.4.1. Fetal and infant outcomes

Maternal vitamin D deficiency predisposes newborns to neonatal hypocalcaemia, and to later rickets. Observational studies also suggest that the bone mass of the newborn is related to the vitamin D status of the mother (reviewed by Salle et al.<sup>131</sup>). In a longitudinal study, it has been reported that children at 9.5 years of age born to vitamin D-deficient (serum 25[OH]D concentration <50 nmol/L) mothers ( $n = 379$ ) had significantly smaller arm-muscle area in comparison with children born to mothers without deficiency ( $n = 189$ ).<sup>132</sup> Moreover, several studies propose low vitamin D concentrations during prenatal or early life development as a cause of a greater risk of later development of multiple sclerosis, cancer, insulin dependent diabetes mellitus, and schizophrenia.<sup>114</sup> There is growing evidence that in rodents vitamin D deficiency during pregnancy may disrupt fetal brain development and may lead to persistent changes in the adult brain.<sup>133</sup> The absence of vitamin D during fetal development on protein expression in the adult brain was studied in pregnant female rats kept vitamin D-deficient until birth.<sup>97</sup> At week 10, protein expression in the progeny's prefrontal cortex and hippocampus was compared with control animals. Developmental vitamin D deficiency caused a dysregulation of 36 brain proteins involved in several biological pathways including oxidative phosphorylation, redox balance, cytoskeleton maintenance, calcium homeostasis, chaperoning, PTMs, synaptic plasticity and neurotransmission. A computational analysis of data revealed that nearly half of the deregulated molecules have also been shown to be misexpressed in either schizophrenia and/or multiple sclerosis. Moreover, in rats a clinical phenotype was induced characterized by hyperlocomotion in the offspring at adult age.<sup>134</sup> A recent meta-analysis shows that the risk of type 1 diabetes is significantly reduced in infants who were supplemented with vitamin D compared to those who were not supplemented suggesting that Vitamin D supplementation in early childhood may offer protection against the development of type 1 diabetes.<sup>135</sup> During pregnancy, few observational studies suggest that vitamin D insufficiency is associated with childhood incidence of type 1 diabetes [adjusted odds ratio (95% CI): 0.74 (0.56, 0.99)].<sup>136</sup> There are also arguments that low maternal vitamin D intake may be associated with the risk of recurrent wheeze at 3 or 5 years which suggests that childhood asthma may be influenced by maternal diet during pregnancy.<sup>137,138</sup> For an example, mothers consuming high daily intakes (median: 724 IU) during pregnancy had a lower risk of having a child with recurrent wheeze at 3 years of age [odds ratio (95% CI): 0.39 (0.25, 0.62),  $p < 0.01$ ] with respect to mothers in the lowest quintile of daily intakes (356 IU).<sup>137</sup> On the whole, the available data suggest that vitamin D status during pregnancy is not only linked to maternal skeletal health and fetal skeletal formation but also may affect maternal outcomes and longer term health of the child,<sup>139</sup> and that the high risk of developing diseases characterized by systemic inflammation may be attributable to vitamin D anti-inflammatory properties.<sup>99</sup> However, there are too few relevant studies to form a strong evidence base, mostly because of small sample size, and because the majority are observational. Further evidence from appropriately designed interventional trials is required. Additional studies are also needed to determine optimal 25[OH]D concentrations with regards not only to bone health but also to non-classical actions of vitamin D on maternal and fetal

outcomes. Vitamin D-deficient or insufficient neonates are at risk of hypocalcaemia and rickets, and recommendations to support an adequate 25[OH]D status of women during pregnancy might thus be best directed toward ensuring vitamin D-sufficiency in the newborn. Unfortunately, there is a lack of consensus on the thresholds of maternal and neonatal plasma 25[OH]D that define the lower limit of adequacy or sufficiency, and international dietary recommendations for dietary vitamin D intake vary considerably. Considering the contrasting opinions on increasing of vitamin D intake recommendations<sup>140</sup> and the limited data on the biological effects, monitoring of effects with higher intakes of vitamin D should be promoted.

#### 2.5. Iron

Neonates at term birth have a total body store of about 270 mg of iron, all derived from the mother. Some of this iron is made available from the stopping of menses, and some from increased absorption during pregnancy. The mother will however have to provide about 400 mg from her own hepatic stores. Moreover, extra iron is required for the formation of the placenta, as well as to expand maternal red cell mass and cope with blood loss during delivery.<sup>141</sup> Women often become pregnant without adequate iron reserves or are already iron deficient. The most severe consequence of iron depletion is maternal iron deficiency anemia (IDA). IDA may be aggravated during pregnancy owing to fetal demands for iron.<sup>142</sup> Iron status seems to be implicated in some pregnancy disorders affecting mother and fetus, such as preeclampsia, prematurity and premature rupture of membranes.<sup>143,144</sup> In a prospective study of 1650 pregnancies, low iron status early in pregnancy was found to be inversely related to placental size.<sup>145</sup> Either anemia in the periconceptional period may have an independent effect on infant growth by influencing hormone synthesis,<sup>146</sup> or the moderate preconceptional anemia may turn to a more severe anemia during pregnancy<sup>142</sup> leading to the observed growth deficits. Animal studies suggest that maternal IDA may be a predictor for increased disease risk in later life,<sup>147</sup> and recently it has been hypothesized that maternal iron status might be involved in cognitive development of the infant.<sup>148</sup> Some studies showed that maternal IDA is linked to altered infant behavioral and neural development, and suggested to result in irreversible effects on neurochemistry and neurobiology due to altered myelination of white matter, changes in monoamine metabolism in striatum and functioning of the hippocampus.<sup>149–151</sup>

##### 2.5.1. Iron supplementation

An adequate iron intake is mandatory for normal fetal growth and development. Nevertheless, an unresolved question is whether prophylactic iron supplementation does reduce the rates of pregnancy complications,<sup>152</sup> or whether anemia affects placental development solely during early pregnancy and therefore would prevent complications by supplementation prior to pregnancy. Routine iron supplementation is still common during pregnancy but potentially harmful effects in iron sufficient women are debated. In general, randomized controlled trials showed that prophylactic iron supplementation during early pregnancy (i.e., 30–40 mg/day taken from the 20th week of gestation until delivery) can increase hemoglobin concentrations and body iron stores in pregnant women.<sup>153–156</sup> Nevertheless, results related to clinical endpoints are conflicting. Some authors reported that supplementation increased birthweight and reduced the incidence of preterm delivery,<sup>152,157</sup> whereas others found no effect<sup>155,158</sup> or negative effects on clinical endpoints, possibly due to untoward effects of iron overload in non-anemic mothers.<sup>133</sup> In this context, Ziaei and colleagues<sup>149</sup> concluded that routine iron



supplementation (50 mg) in non-anemic women was not rational and might be harmful. Their randomized controlled trial evidenced that small-for-gestational-age (SGA) birth rate and the number of women with hypertension disorders increased significantly in the intervention in comparison with the control group, being 15.7% vs 10.3%,  $p = 0.035$ , and 2.7% vs 8%,  $p = 0.05$ , respectively. The dosage of iron is a critical issue in relation to potential side effects. In excess, iron may be toxic because of its ability to generate reactive oxygen species and to induce cell and tissue damage.<sup>159</sup> During pregnancy, increased numbers of mitochondria lead to increased synthesis of unpaired electrons and therefore may contribute to oxidative stress, with a peak vulnerability during the second half of pregnancy for fetal health and gestational outcomes.<sup>160</sup> A recent observation study reported a 2-fold higher prevalence of GDM, hypertension, and metabolic syndrome in mid-pregnancy among iron-supplement users,<sup>161</sup> suggesting that iron excess should be avoided.<sup>160</sup>

However, iron deficiency anemia should certainly be prevented and treated. Selective prophylaxis seems to be the most effective and appropriate approach: screening of ferritin levels in early

pregnancy may identify women who may benefit from iron supplementation.

Ongoing studies are evaluating the effect of different timing of iron supply in pregnancy (i.e. early or late pregnancy) as well as the different frequency of supplementation (i.e., daily or weekly). Moreover, different forms of iron supplements are of interest given that iron status is influenced both by the iron content and the bioavailability of iron: indeed heme iron from animal foods is better absorbed than non-heme iron from vegetables. Similarly FeEDTA and liposome embedded/encapsulated forms may have a higher bioavailability than ferrous fumarate or FeSO<sub>4</sub>.<sup>162–164</sup> Polymeric forms may have advantages because of a lesser likelihood to be reactive. The further exploration of different strategies could inform future practice recommendations.

## 2.6. Iodine

Approximately half of the European population is still suffering from an inadequate iodine supply. Low urinary iodine excretion is especially common among pregnant women and school children.

**Table 3**

Micronutrient dietary sources, dietary reference intakes for pregnant women, unresolved issues and proposal of priority for future research in pregnancy.

Micronutrient	Dietary source	Dietary Reference Intakes (DRIs)	Issues	
			To be re-assessed	Priority for future research
Folate (DEF) (µg/day)	Enriched cereal grains, dark leafy vegetables, enriched and whole-grain breads and bread products, fortified ready-to-eat cereals	600 (FAO/WHO) <sup>11</sup> ; 600 (IOM) <sup>84</sup> ; 300 (COMA, UK) <sup>85</sup> ; 600 (D-A-CH) <sup>86</sup> ; 600 (Australia) <sup>103</sup> ; 500 (NNR) <sup>177</sup>	400 <sup>a</sup> from 4 wks before until 12 wks after conception <sup>21</sup>	Dose/timing combination: 400 starting 8–12 wks before pregnancy or 800 starting 4 wks before pregnancy. Investigation on toxicity of chronic high doses
Vitamin A (retinol equivalent) (mg/day)	Liver, dairy products, fish, darkly colored fruits, leafy vegetables	0.8 (FAO/WHO) <sup>11</sup> ; 0.77 <sup>b</sup> (IOM) <sup>84</sup> ; 0.7 (COMA, UK) <sup>85</sup> ; 1.1 (D-A-CH) <sup>86</sup> ; 0.8 (Australia) <sup>103</sup>	To avoid intake of vitamin A rich liver and liver foods during pregnancy	To investigate effects of higher intakes of vitamin A during the 3rd trimester of gestation
Vitamin D (µg/day)	Fish liver oils, flesh of fatty fish, liver, eggs, fortified milk products, fortified cereals	5 (FAO/WHO) <sup>11</sup> ; 10 (UK) <sup>104</sup> ; 15 (IOM) <sup>105</sup> ; 5 (D-A-CH) <sup>86</sup> ; 5 (Australia) <sup>103</sup>	Optimal dose of Vitamin D to achieve sufficiency.	To determine optimal 25[OH]D concentrations with regards to bone health and non-classical actions of vitamin D on maternal and fetal outcomes. Need of selecting high risk population for routine supplementation during/before pregnancy.
Iron (mg/day)	Fruits, vegetables, fortified bread and grain products such as cereal (nonheme iron), meat and poultry (heme iron)	27 (IOM) <sup>84</sup> ; 14.8 (COMA, UK) <sup>85</sup> ; 30 (D-A-CH) <sup>86</sup> ; 27 (Australia) <sup>103</sup>	Routine iron supplementation	Selective prophylactic supplementation. To avoid overload. To evaluate the effects of timing and frequency of supplementation, and to investigate the bioavailability of different forms of supplements
Iodine (µg/day)	Marine origin, processed foods, iodized salt	220 (IOM) <sup>84</sup> ; 140 (COMA, UK) <sup>85</sup> ; 230 (D-A-CH) <sup>86</sup> ; 220 (Australia) <sup>103</sup> ; 200–250 (WHO) <sup>166</sup> ; 175 (NNR) <sup>177</sup>	Supplementation of 100–150 µg Iodine in countries with a mean iodine uptake below 150 µg/day	To control urinary iodine (UI) excretion by epidemiological studies under the current push to reduce salt intake in the general population
ANTIOXIDANTS Selenium (µg/day) (including selenoproteins)	Bread and cereals, meat, milk/dairy <sup>178</sup>	28 (2nd trimester) – 30 (3rd trimester) (FAO/WHO) <sup>11</sup> ; 60 (IOM) <sup>84</sup> ; 60 (COMA, UK) <sup>85</sup> ; 65 (Australia) <sup>103</sup> ; 55 (NNR) <sup>177</sup>	Circumstantial evidence that selenium supplements may prevent preeclampsia	To investigate other more specifically targeted antioxidant strategies for prevention of preeclampsia including an RCT of selenium supplementation in low selenium populations

<sup>a</sup> DEF = Dietary Folate Equivalents.

<sup>b</sup> RAE = Retinol Activity Equivalent.

During pregnancy the demand for iodine is substantially higher than in non-pregnant women increasing from at least 100 µg/day to about 200–250 µg/day to maintain free thyroxine (fT4) during pregnancy within the normal range. This is because of the increased demand for thyroxine during the first trimester, the increase in thyroxine-binding protein and the higher loss of iodine in the urine.<sup>165</sup> The WHO recommendation for iodine intake during pregnancy and lactation has recently increased from 200 to 250 µg/day and suggested that a median urinary iodine concentration (UIC) of 150–250 µg/L indicates adequate iodine intake.<sup>166</sup> WHO recommends iodine supplementation in pregnancy only in countries where less than 90% of households use iodized salt or where the median UIC in school children is below 100 µg/L. However, the use of iodized salt in households might be not enough to cope with the increased iodine demand during pregnancy and therefore the American Thyroid Association recommends an iodine supplementation of 150 µg/day in pregnant women.<sup>167</sup>

Low iodine intake in pregnancy is associated with a higher incidence of miscarriage, SGA infants, and a delay in mental development or even permanent mental retardation, reduced intellectual ability and hearing loss.<sup>166</sup> Thyroxine is necessary for normal brain development<sup>168</sup> and therefore a sufficient iodine intake especially within the first trimester is important.<sup>169</sup> Even mild iodine deficiency and subclinical hypothyroidism may affect fetal growth. Pregnant women with UICs during the 3rd trimester below 50 µg/L were significantly more likely to have a SGA infant, and mean birthweight was lower, than among women with the UICs between 100 and 149 µg/L [adjusted odd ratio (95%CI): 0.15 (0.03–0.76)]. Higher TSH levels were also associated with a higher risk of having an SGA baby or a LBW newborn.<sup>170</sup> The later mean intelligence quotient of children born to women an UIC below 50 µg/L was found significantly lower compared to controls with adequate iodine supplementation.<sup>171</sup> Recent cross-sectional studies in European countries and the USA show a median UIC in pregnant women in the range of 95–130 µg/L.<sup>166,172</sup>

Excessive iodine intake like in Japan did not affect either the maternal or the off-spring thyroid function.<sup>173</sup> Other studies show an increase in iodine-induced hyperthyroidism in areas with previously low iodine intake and also a higher prevalence of autoimmune thyroiditis (AIT) is discussed.<sup>166</sup> Therefore, it has been suggested to test the thyroid function of women who plan a pregnancy in geographical areas with known iodine deficiency, but this concept is controversial.<sup>174</sup>

During the 1st trimester the fetus depends on the thyroxine supplied by the mother, and thereafter mainly on maternal iodine intake. The maternal fT4 concentration therefore is more important for fetal brain development than TSH, although elevated TSH may indicate a deficient thyroid reserve of the mother and AIT should then be excluded.<sup>168,172</sup> If AIT is confirmed, L-Thyroxin supplementation is indicated to keep TSH and fT4 within the low normal range. After the 12th week of gestation, iodine should be added in a dosage of 150 µg/day. If AIT is excluded, only iodide supplementation (150–200 µg/day) is recommended throughout pregnancy and lactation in several countries with frequent occurrence of iodine deficiency (e.g. Germany), but obviously the iodine dose supplied to prevent and treat iodine-deficiency disorders should be at a safe level. If iodine status is good (median UIC, 200–299 µg/L) or excessive (median UIC, >300 µg/L) additional supplementation should be avoided. Iodized salt should be provided only in regions where iodine intake is insufficient according to the WHO recommendations.

The thyroid is the organ with the body's highest selenium content. Combined iodine and selenium deficiency is the cause of myxoedematous cretinism. This is because selenium-dependent enzymes (GPx's) are important to prevent the thyroid from

oxidative damage.<sup>175</sup> Sufficient dietary iodine and selenium intakes are necessary for normal thyroid function and low selenium intake might initiate or exacerbate AIT. Selenium supplementation in countries with low selenium intake might attenuate the inflammatory activity within the thyroid in patients suffering from AIT. Supplementation of 200 µg selenium per day during pregnancy and 9 months after delivery in women suffering from AIT was reported to prevent the post-partum exacerbation of AIT and decreasing the incidence of post-partum hypothyroidism.<sup>176</sup> This study, however, needs confirmation especially in countries with sufficient selenium intake.

### 3. Conclusions

In addition to their classical essential roles, current evidence seems to suggest that vitamins and minerals have added bio-functionality which may be particularly important in pregnancy. In particular, besides meeting desirable intakes, the balance between antioxidants and pro-oxidants needs to be sustained. For example supplementation with iron should not be excessive in order to avoid adverse outcomes. Moreover, synergisms between different micronutrients may enhance their biological potential, for example, the combination of folic acid and vitamin B<sub>12</sub> appears to further reduce the risk of occurrence of NTDs.

In this context, more information on the biological mechanisms underpinning relationships between micronutrients and pregnancy outcomes is needed. Moreover, discrepancy between results from observational studies and randomized controlled studies often exists (i.e., role of folic acid in carcinogenesis), and data from trials are often conflicting as well, as suggested for example by the disagreement on the need for iron prophylaxis or on the harmful amount and timing of vitamin A intake (Table 3). Similarly, large trials evidenced no effect of the prophylactic use of high doses of vitamin C and vitamin E combined on the prevention of preeclampsia. Moreover, it should be investigated whether the strategy of increasing folic acid dose from 400 to 800 µg/day may be applicable to pregnancy state where adaptation happens to increase absorption of nutrients. Vitamin D insufficiency is increasingly common amongst pregnant women and the consequences may extend beyond the recognized influence of neonatal calcium deficiency and rickets. The proposed relationships between hypovitaminosis and gestational diabetes, and with preeclampsia, require further investigation, as does the influence of maternal supplements required to achieve sufficiency. Adequately powered, randomized controlled trials with long periods of follow-up are needed to establish causality and the best formulation, dose, duration and period of supplementation during pregnancy. The influence on the health of the child in later life should routinely be considered. Clearly, further studies are needed to determine the optimal 25[OH]D concentrations with regards not only to bone health but also to non-classic actions of vitamin D on maternal and fetal outcomes. The benefit of iodine for the brain development of the offspring during pregnancy is well established. The main source of iodine is the use of iodized salt in household and food industry. The current recommendations to reduce salt intake to prevent hypertension might influence iodine uptake of women in child-bearing age and therefore especially also in developed countries surveys of iodine intake is recommended and increase of the amount of iodine in fortified salt has to be considered.

Micronutrient deficiencies can undoubtedly have profound influences on the health of the mother and her child, but they remain areas of uncertainty and controversy which hinder the development of robust public health guidance. Many of these, addressed in the review, would be clarified by well conducted randomized controlled clinical trials including childhood follow-

up. These are an essential pre-requisite to inform evidence based policy decisions and recommendations for clinical practice.

### Conflict of interest

The authors do not report a potential conflict of interest.

### Statement of authorship

We hereby certify that it is an original publication, the manuscript has not been previously submitted or published elsewhere. CB prepared the first draft of the manuscript, and HB, RG, AL, KP, LP, CR, BK and IC contributed to writing the final version of manuscript. All authors have made substantial contributions to and approved the final version of the manuscript.

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