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# Cobalamin status in children

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**Abstract** Cobalamin and the metabolic markers methylmalonic acid and total homocysteine undergo marked changes during childhood. In breastfed infants a metabolic profile indicative of cobalamin deficiency is common. Symptoms of cobalamin deficiency in children differ with age, presenting a continuum from subtle developmental delay to life-threatening clinical conditions. The symptoms may be difficult to detect, particularly in infants, and there tends to be a diagnostic delay of several months in this age group. Several reports show that even moderate deficiency in children may be harmful, and long-term consequences of neurological deterioration may persist after cobalamin deficiency has been treated. Given the crucial role of cobalamin for normal growth and development, possible widespread infantile deficiency needs attention. Cobalamin deficiency should be considered a differential diagnosis in children with subtle symptoms, and strategies to prevent cobalamin deficiency in mothers and children should be addressed.

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**Abbreviations**

| | |
|---|---|
| BMI | body mass index |
| methylmalonyl CoA mutase | methylmalonyl coenzyme A mutase |
| MMA | methylmalonic acid |
| MRI | magnetic resonance imaging |
| PPI | proton-pump inhibitors |
| RDA | recommended dietary allowances |
| tHcy | total homocysteine |
| WHO | World Health Organization |

## Introduction

Cobalamin deficiency is a worldwide problem, especially in developing countries where deficiency is common in all age groups (Stabler and Allen 2004; Allen 2009). In developed countries, cobalamin deficiency is most common at advanced age; however, the prevalence in younger age groups may be higher than formerly recognized (Honzik et al. 2010; Monsen et al. 2003; Allen 2009).

## Cobalamin during growth and development

Several reviews have outlined the importance of adequate maternal vitamin B status for a normal pregnancy (Black 2008; Finnell et al. 2008; Molloy et al. 2008). Maternal cobalamin deficiency is associated with infertility, early pregnancy loss (Bennett 2001), increased prevalence of preeclampsia, preterm birth (Bondevik et al. 2001; George et al. 2002), and a moderate risk of neural tube defects (Kirke et al. 1993; Ray

and Blom 2003). Throughout childhood, an adequate cobalamin status is important for normal growth and development, as demonstrated by the clinical picture presented in children with inborn errors of cobalamin absorption, transport, and metabolism (Rosenblatt and Whitehead 1999; Whitehead 2006; Fowler et al. 2008). The symptoms differ according to age at onset and severity. In infants, neurological impairment with hypotonia, seizures, developmental delay, and brain atrophy are typical, and the damage is thought to occur during the first 6 months, which is a critical period for maturation of oligodendrocytes and brain myelination (Lovblad et al. 1997). In severe cases, the patients die during early childhood. In older children, megaloblastic hematological abnormalities, extrapyramidal signs, dementia, delirium, or psychosis are observed (Whitehead 2006).

## Possible mechanisms behind the central nervous system lesions

The mechanisms behind brain dysfunction are not fully understood but may be related to the function of the two cobalamin-dependent enzymes, methionine synthase and methylmalonyl coenzyme A (CoA) mutase, which require methylcobalamin and adenosylcobalamin, respectively, as cofactors. Impaired methionine synthesis may reduce the supply of S-adenosylmethionine necessary for methylation reactions in the nervous system (Weir et al. 1988, 1990), whereas low methylmalonyl CoA mutase activity causes an accumulation of methylmalonate and propionate, leading to the synthesis of abnormal fatty acids. The incorporation of these fatty acids into myelin, especially in the spinal cord, may cause defective myelination and thereby the central nervous system (CNS) lesions observed in cobalamin deficiency (Hall 1990).

## Age-related changes in cobalamin status during childhood

Cobalamin, folate, and the metabolic markers total homocysteine (tHcy) and methylmalonic acid (MMA), undergo marked changes during childhood (Monsen et al. 2003) (Fig. 1). During the first weeks of life, there is a considerable decrease in serum cobalamin level, accompanied by a marked increase in plasma tHcy and MMA (Minet et al. 2000; Bjorke Monsen et al. 2001; Fokkema et al. 2001). The lowest cobalamin levels and the highest tHcy and MMA levels in childhood are seen in infants 6 weeks to 6 months of age. In older children (>6 months), serum cobalamin increases and peaks at 3–7 years and then decreases, median plasma tHcy remains low (<6 μmol/L) and increases from the age of 7 years, whereas median

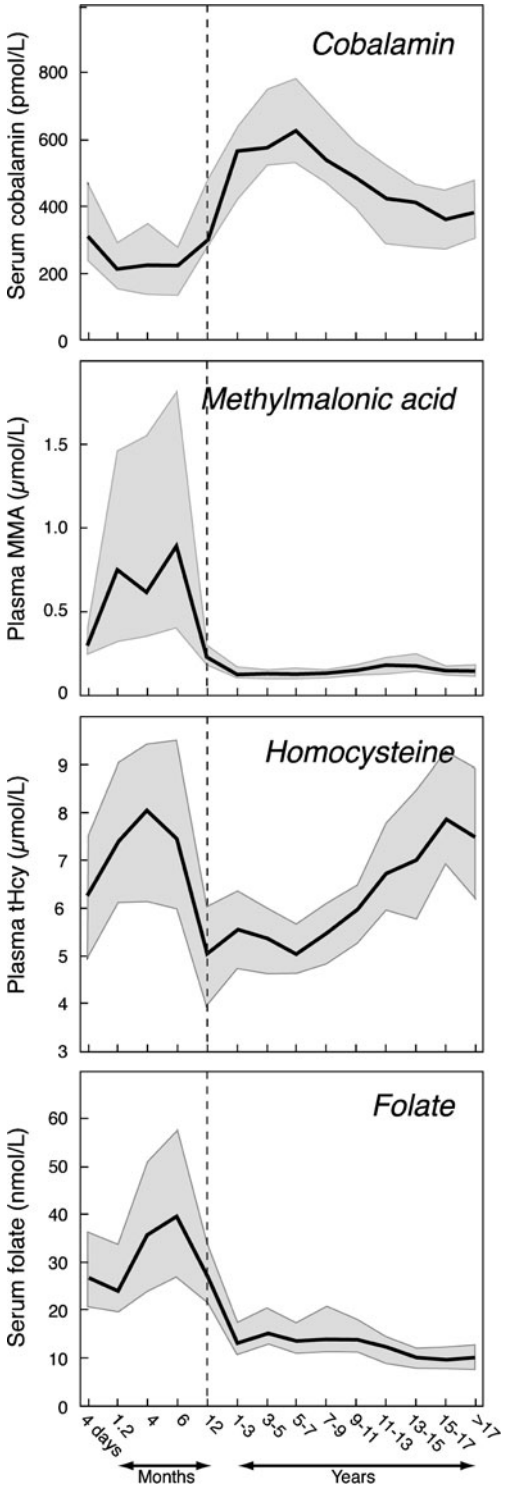


**Fig. 1** Changes in serum cobalamin and folate and plasma total homocysteine (tHcy), and methylmalonic acid (MMA) in children from day 4 throughout adolescence. The *solid lines* indicate the values; *shaded areas* indicate the 25th and 75th percentiles. Reprinted with permission from Monsen et al. (2003)

plasma MMA remains low throughout childhood (<0.26 μmol/L) (Monsen et al. 2003).

Associations between the B vitamins, cobalamin and folate, and the metabolic markers tHcy and MMA differ between infants and older children. In the first year of life, plasma tHcy is strongly correlated to serum cobalamin, whereas the relation to serum folate is weak or absent (Minet et al. 2000; Bjorke Monsen et al. 2001; Guerra-Shinohara et al. 2002; Molloy et al. 2002). In infants ≤6 months, MMA is inversely related to cobalamin, but the MMA concentrations are higher through the range of cobalamin concentrations than in older children (Monsen et al. 2003). The cause of the higher MMA levels in breastfed infants is uncertain, but it may be the result of increased intestinal absorption of propionate and MMA precursors produced by intestinal bacteria or degradation of odd-chain fatty acids present in breast milk (Refsum et al. 1991; Magera et al. 2000; Monsen et al. 2003; Molloy et al. 2008). In older children and adults (≥24 mo), plasma tHcy shows a stronger association with folate than with cobalamin (De Laet et al. 1999; Osganian et al. 1999; Delvin et al. 2000; Monsen et al. 2003).

## Cobalamin deficiency: occurrence and children at risk

Cobalamin is a water-soluble vitamin synthesized by bacteria and algae. Dietary cobalamin is derived exclusively from animal sources, such as meat, fish, and dairy products, or from foods that have been fermented. Plants usually neither synthesize nor accumulate cobalamin, but some plants (peas and beans) may under certain circumstances be able to synthesize small amounts (Castle and Hale 1998). Populations that consume large amounts of animal products ingest 3–22 μg/day of cobalamin compared with 0–0.25 μg/day for strict vegetarians (Allen 1994). Recommended dietary allowances (RDA) from the Institute of Medicine 1998 are presented in Table 1. A recent study has disputed the current RDA of 2.4 μg/day for adults, showing that in a healthy population aged 18–50 years, a cobalamin intake of 4–7 μg/day is associated with an adequate biochemical cobalamin status (Bor et al. 2010).

There is, however, no clear agreement about what constitutes a normal cobalamin status, and various cutoff levels for cobalamin, tHcy, and MMA indicating deficiency have been considered (Fedosov 2010; Scott 1999; Carmel et al. 2003). It has been suggested that normal ranges of tHcy and MMA can be obtained by measuring these markers in vitamin-replete individuals after vitamin supplementation (Ubbink et al. 1995). Interpretation of cobalamin, tHcy, and MMA levels requires caution, as not only vitamin status, but also physiological changes, such as pregnancy (Murphy et al. 2007), age, gender, and renal function (Rasmussen et al. 1996; Bjorke Monsen and Ueland 2003; Monsen et al. 2003; Schneede et al. 2003; Vogiatzoglou et al. 2009), may affect blood concentrations. A low cobalamin level may be due to haptocorrin deficiency and does not necessarily indicate vitamin deficiency (Carmel 2000). In infants, cobalamin deficiency is mainly due to maternal depletion of the vitamin, and in older children and adults, a low intake of animal products is the main cause. Deficiency due to malabsorption increases with increasing age; in children, a common cause is intestinal parasite infection, whereas pernicious anemia is rare (Stabler and Allen 2004). Cobalamin dysfunction due to inborn errors of cobalamin absorption, transport, and metabolism is very rare (Whitehead 2006).

**Table 1** Recommended dietary allowance (RDA) for cobalamin in children and adults

| Target group | Age (years) | Dose (μg) |
|---|---|---|
| Children | <0.5 | 0.4 |
| | 0.5–1 | 0.5 |
| | 1–3 | 0.9 |
| | 4–8 | 1.2 |
| | 9–13 | 1.8 |
| | 14–18 | 2.4 |
| | >19 | 2.4 |
| Pregnant women | | 2.6 |
| Lactating women | | 2.8 |

Based on data from the Institute of Medicine (1998)

### Fetal life

It has been estimated that the human fetus accumulates 0.1–0.2 μg cobalamin per day. Infants of well-nourished women have about 25–30 μg of cobalamin stored in the liver and a total body content of 50 μg at birth (Allen 1994). Premature and low-birth-weight infants have lower levels compared with full-term infants with normal birth weight (Pathak and Godwin 1972; Baker et al. 1977). In the United States, the usual dietary intake of cobalamin during pregnancy is about 2.6–6.6 μg/day (Institute of Medicine 1990). Based on these figures, pregnancy seems to place relatively little drain on maternal cobalamin stores in well-nourished women. However, a longitudinal study of healthy Danish women demonstrated increasing levels of the metabolic markers tHcy and MMA during pregnancy and lactation, indicating that low cobalamin status may occur among pregnant women in developed countries (Milman et al. 2006). An increase in MMA levels during pregnancy is observed in women with normal preconceptional cobalamin levels, and the increase being greater in the group with lower holotranscobalamin (holoTC). This indicates that pregnancy causes a strain on cobalamin status (Murphy et al. 2007). Cobalamin deficiency in childbearing or breast-

feeding women may be due to malabsorption due to gastric surgery, short gut syndrome, intestinal parasite infection, or unrecognized early pernicious anemia, but the most common cause is a low cobalamin diet, or vegetarianism (Rosenblatt and Whitehead 1999). Cobalamin depletion can result from consumption of such diets for as little as 3 years (Rosenblatt and Whitehead 1999). Additionally, both parity (Bjorke Monsen et al. 2001), and maternal smoking during pregnancy (Frery et al. 1992), have been associated with reduced cobalamin status in the mother and in the neonate.

## Infancy

An inadequate cobalamin status in a newborn may be further deteriorated by a low cobalamin content in breast milk. Exclusive breastfeeding for extended periods places nutritional demands on the mother (Kontic-Vucinic et al. 2006). The cobalamin content of human milk reflects the maternal cobalamin concentration in blood (Specker et al. 1990a; Black et al. 1994; Ford et al. 1996), and it falls progressively during the lactation period (Craft et al. 1971; Sneed et al. 1981; Patel and Lovelady 1998). The reduced cobalamin content is thought to be compensated by increasing milk output to the child (Craft et al. 1971). However, Ford et al. (1996) reported that the estimated cobalamin intake of the infant (based on a daily milk intake of 150 ml/kg body weight) was maximal at 12 weeks and reduced to about 50% at 24 weeks. The reported content of cobalamin (150–700 pmol/L) in human milk may be influenced by the haptocorrin content in the milk (Lildballe et al. 2009) and is related to the assay used (Specker et al. 1990a; Ford et al. 1996). Most commercially prepared infant formulas are enriched with cobalamin up to concentrations of 800–1,200 pmol/L (Ford et al. 1996). Consequently, infants fed formula obtain higher amounts of cobalamin compared with breastfed infants and have higher serum levels of cobalamin and lower levels of tHcy and MMA (Specker et al. 1990a; Minet et al. 2000). The World Health Organization (WHO) recommended in 2001 that infants should be exclusively breastfed for the first 6 months of life (World Health Organization 2001). Exclusive breastfeeding for extended periods is associated with both short- and long-term feeding difficulties (Northstone et al. 2001; Coulthard et al. 2009), which may postpone weaning, thereby increasing the risk of cobalamin deficiency. In some cases, infant refusal to breastfeeding necessitates introduction of bottle feeding with formula, which improves food acceptance and eventually enhances cobalamin status due to the higher cobalamin content in formula compared with human milk (Specker et al. 1990a; Minet et al. 2000). Concerns have been raised that there are insufficient evidence to support the WHO recommendation (Butte et al. 2002; Fewtrell et al. 2007). The need for supplemental vitamin K and D in exclusively breastfed infants demonstrates that human milk is not a complete food (Collier et al. 2004).

In recent years, an increasing number of severely affected cobalamin-deficient infants have been diagnosed (Molloy et al. 2008), and this has been attributed to exclusive breastfeeding for extended periods of time. Impaired cobalamin status that could be corrected by cobalamin supplementation was observed in more than two thirds of mainly breastfed Norwegian infants aged 6 weeks to 4 months (Bjorke-Monsen et al. 2008). The infants given cobalamin had higher cobalamin levels and lower tHcy, MMA, and folate levels compared with controls at 4 months. It is unlikely that cobalamin supplementation could reverse this biochemical pattern if it was due to organ immaturity. Methionine synthase catalyzing the conversion of homocysteine to methionine is, under normal conditions, fully saturated with its cobalamin cofactor and is not stimulated by exogenous cobalamin (Chen et al. 1995). A reduction of tHcy by cobalamin supplementation strongly suggests the presence of impaired cobalamin status. Notably, the lower serum folate in the intervention group indicates reversal of the so-called methylfolate trap. In early cobalamin deficiency, serum 5-methyltetrahydrofolate levels are reported to increase due to inhibition of the cobalamin-dependent enzyme methionine synthase (Allen et al. 1993). The observation of high tHcy and MMA in a large proportion of infants indicates that cobalamin deficiency may be common among full-term infants in developed countries (Specker et al. 1990b; Minet et al. 2000; Monsen et al. 2003). Whether cobalamin supplementation will have a clinical impact in this age group has yet to be evaluated in cobalamin intervention studies, which include neurodevelopmental evaluation of the child.

## Childhood and adolescence

A high prevalence of cobalamin deficiency in the range of 40–80% has been found in both children and adults in developing countries (Allen 2004; McLean et al. 2007; Taneja et al. 2007), whereas in Spanish children aged 2–6 years, cobalamin intake was more than four times the RDA, and only 2.9% had an intake below the recommendation (Requejo et al. 1997). However, in developed countries, diet quality tends to decrease during preadolescence (Mannino et al. 2004), and adolescence is considered a particularly nutritionally vulnerable period, characterized by rapid growth and often great changes in dietary habits. Data from the Third National Health and Nutrition Examination Survey (1988–1994) (NHANES III, show that >95% of US children consume more than the daily cobalamin requirement of 0.4–2.4 μg (Audelin and Genest

2001), but the lowest cobalamin levels are seen in children aged 12–19 years, with one out of 112 children having a cobalamin level <200 pg/ml (Wright et al. 1998).

In the early twentieth century, few people considered vegetarian foods as sufficient for a healthy diet (Whorton 1994). Current dietary guidelines propose shifts in food composition toward more vegetarian than omnivore patterns in order to reduce energy intake and cholesterol levels (Obarzanek et al. 1997). There has recently also been an increasing interest in and use of alternative diets, especially among young people. In a Swedish study, about 5% of adolescents aged 16–20 years ate a vegetarian lunch and 0.1% ate only vegetarian food (Larsson and Johansson 1997). The vegetarian diet was very heterogeneous and associated with a lower-than-recommended intake of cobalamin (Larsson and Johansson 2002). A low cobalamin status for several years may be difficult to restore merely by dietary changes. Moderate consumption of animal products for several years was not sufficient for restoring normal cobalamin status in adolescents fed a macrobiotic diet until 6 years of age (van Dusseldorp et al. 1999).

### Pharmacological agents associated with cobalamin deficiency

Gastroesophageal reflux is especially prevalent in infants but occurs also in older children. Preferred pharmacological treatment to decrease acid secretion in children include H2-receptor antagonists and proton-pump inhibitors (PPI) (Indrio et al. 2009). Decreased gastric acid secretion reduces cobalamin release from dietary proteins, and long-term use may lead to cobalamin deficiency (Ruscin et al. 2002). However, two studies in children observed no cobalamin deficiency among patients treated with PPI for 24–35 months (ter Heide et al. 2001; Tolia and Boyer 2008) There has been an increasing use of metformin in children, mainly due to the worldwide increase of type 2 diabetes in young people (Kempf et al. 2008). Metformin is also used to reduce body mass index (BMI) and insulin resistance in hyperinsulinemic obese children and adolescents (Park et al. 2009). In adults, metformin is associated with decreased cobalamin levels (Leung et al. 2010) and elevated tHcy and MMA levels (Wile and Toth 2010). To our knowledge, no data has been published on cobalamin deficiency in children on such medication, but the risk for cobalamin deficiency should be of concern in this age group also.

## Symptoms of cobalamin deficiency in infants and older children

Symptoms and the long-term prognosis of cobalamin deficiency in children differ with age and depend on the severity and duration of deficiency (Rosenblatt and Cooper 1990). It is a continuum of symptoms, from subtle developmental delay in small infants to life-threatening clinical conditions. With severe maternal cobalamin deficiency, symptoms may be evident at birth; however, in most patients, symptoms are manifest by the age of 4–8 months (Allen 2002). As many doctors are not familiar with the symptoms of cobalamin deficiency in children, there tends to be a diagnostic delay (Dror and Allen 2008; Zengin et al. 2009). In a review of 48 published cases of infantile cobalamin deficiency, there was a median diagnostic delay of 4 months after the first symptom appeared (Dror and Allen 2008). The diagnosis is particularly challenging in infants, where symptoms may be nonspecific and difficult to detect, partly due to the large variation in normal development in this age group (Casella et al. 2005; Cetinkaya et al. 2007). In infants, cobalamin deficiency may present as failure to thrive, feeding difficulties, refusal of complementary food, dysphagia, obstipation, regurgitation, and vomiting (Cetinkaya et al. 2007; Dror and Allen 2008; Zengin et al. 2009). Developmental delay is common, and in severe cases, even regression may occur. Neurological examination may reveal hypotonia, insufficient head control, and delayed turning, sitting, and walking. Drowsiness, reduced eye contact and smiling, and apathy or irritability may be evident (Dror and Allen 2008; Zengin et al. 2009). Abnormal movements such as tremors, twitches, chorea, or myoclonus are commonly observed (Emery et al. 1997; Grattan-Smith et al. 1997). These movements usually disappear with treatment but may appear several days after start of treatment when the child is improving clinically (Yavuz 2008). Cranial magnetic resonance imaging (MRI) in infantile cobalamin deficiency has revealed signs of delayed myelination and frontoparietal cortical atrophy (Lovblad et al. 1997; Avci et al. 2003).

Due to the considerable physiological changes in hematological indices during the first year of life, the diagnostics in this age group is complicated. In the majority of reported cases with infantile cobalamin deficiency, which has been severe or longstanding, the infants have had megaloblastic anemia with mean corpuscular volume (MCV) ranging from 104–117 fL (Dror and Allen 2008; Zengin et al. 2009). Infants with marginal cobalamin status may have neurological symptoms without any hematological abnormalities (Honzik et al. 2010; Monsen et al. 2003). Brownish black hyperpigmentation affecting dorsal fingers and toes and medial thighs, arms, and axilla have been reported in children with severe cobalamin deficiency (Baker et al. 1963; Gilliam and Cox 1973; Sabatino et al. 1998; Simsek et al. 2004). The hyperpigmentation, mostly reported in darker-pigmented children, usually resolves completely within 2–12 weeks of treatment (Heath and Sidbury 2006).

Severe cobalamin deficiency is associated with growth retardation, affecting weight, length, and head circumfer-

ence (Graham and Meleady 1996; Dror and Allen 2008). Children raised on a macrobiotic diet tend to be small; at a median age of 4 years, 32% were below the fifth percentile for height and 15% were below the fifth percentile for weight (Dagnelie et al. 1985). However, the child may have a normal weight and may even be obese despite the serious malnutrition (Wighton et al. 1979).

## Treatment

As cobalamin deficiency in children is mainly due to low dietary cobalamin, oral therapy is more relevant in a pediatric setting than in older persons. Mild deficiency can be treated by introducing animal food into the diet or by orally administered cobalamin supplements. In infants with moderate cobalamin deficiency (median serum cobalamin 170 pmol/L; median plasma tHcy 7.46 μmol/L), 400 μg hydroxocobalamin given i.m. at 6 weeks was associated with a normal cobalamin status at 4 months (median serum cobalamin 420 pmol/L; median plasma tHcy 4.57 μmol/L) (Bjorke-Monsen et al. 2008). This dose represents approximately twice the RDA of cobalamin for the first year of life (Table 1) and has been associated with increased hemoglobin levels (Worthingtonwhite et al. 1994) and reduced tHcy and MMA levels in premature infants (Bjørke-Monsen et al., unpublished observations 2003). In severe cobalamin deficiency, different regimes have been proposed (Rasmussen et al. 2001). Cobalamin may be given as i.m. injections of cyano- or hydroxocobalamin in doses ranging from 250 to 1000 μg from three to seven times per week for 1–2 weeks, then once weekly for 1 month or until blood count is normal. Maintenance doses every 2–3 month are necessary if the underlying condition is not corrected. (Stollhoff and Schulte 1987; McPhee et al. 1988; von Schenck et al. 1997; Ozer et al. 2001; Rasmussen et al. 2001). In severe anemia, low initial doses (0.2 μg/kg for the first 2 days) and potassium supplementation are recommended to prevent hypokalemia (Rasmussen et al. 2001).

Involuntary movements are commonly observed during cobalamin therapy (Benbir et al. 2007; Yavuz 2008). Although variations in the timing are described, movements often appear a few days after initiation of cobalamin injections (Garewal et al. 1988; Emery et al. 1997; Avci et al. 2003; Benbir et al. 2007; Yavuz 2008). Movements are described as a combination of tremor and myoclonus and may affect extremities as well as face and tongue, making feeding difficult. Movements are reported to regress spontaneously within 3–6 weeks (Benbir et al. 2007).

An immediate response to cobalamin treatment is reported in symptomatic infants (Stollhoff and Schulte 1987; McPhee et al. 1988; von Schenck et al. 1997; Ozer et al. 2001; Rasmussen et al. 2001). There is an instantaneously enhanced responsiveness and alertness, and children who have been comatose regain consciousness within hours after the first cobalamin injection. Skeletal muscle hypotonia almost immediately disappears, but in some cases, it may persist for several weeks. There is a remarkable improvement in appetite, and the child starts to eat and drink normally within days (Dror and Allen 2008); within months, the child assumes normal growth parameters, including a normal head circumference (Graham et al. 1992). Hematological changes are rapid, with reticulocytosis appearing after a few days; within a few weeks, peripheral blood values and the bone marrow have gained a normal pattern (Jadhav et al. 1962; Wighton et al. 1979; Stollhoff and Schulte 1987). The apparent morphological changes in the nervous system resolve rapidly (Surtees et al. 1991; Lovblad et al. 1997). Atrophy of the optic nerve resolved completely after 6 months of treatment (Kühne et al. 1991) and cerebral atrophy after 10 weeks (Stollhoff and Schulte 1987; von Schenck et al. 1997).

## Conclusion

Cobalamin deficiency seems to be common among children all over the world. In infants, the diagnosis may be difficult, and there tends to be substantial diagnostic delay (Dror and Allen 2008). Although early response to cobalamin treatment is satisfying and morphological changes in the nervous system detectable by MRI disappear (Surtees et al. 1991; Lovblad et al. 1997), long-term consequences of neurological deterioration may persist after cobalamin deficiency has been treated. Graham et al. reported a poor intellectual outcome of two of four children treated for cobalamin deficiency at 8 and 14 months of age (Graham et al. 1992), and psychomotor retardation in children adequately treated in infancy has also been observed by others (Stollhoff and Schulte 1987; von Schenck et al. 1997). In children from macrobiotic families, evidence of impaired cognitive performance was demonstrated, even in adolescence, after consumption of animal products from the age of 6 years (Louwman et al. 2000). This emphasizes the importance of cobalamin status for CNS development and shows that even moderate deficiency in children may be harmful.

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