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The effect of folinic acid supplementation on homocysteine concentrations in newborns

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Abstract

Objective:

The incidence of cerebrovascular accidents (CVA) occurring perinatally is relatively high and aspects of the multifactorial pathophysiology remain unclear. Elevated homocysteine concentrations have been shown to be associated with an increased risk for CVA in children and even in newborns. We studied the possible homocysteine lowering effect of folinic acid in newborns.

Method:

We included 37 newborns in our prospective randomized folinic acid (given as 5-formyltetrahydrofolate) intervention study from patients admitted to our Neonatal Intensive Care Unit (18 controls, 19 intervention group). We measured total homocysteine (tHcy) and plasma folate concentrations at three time points (baseline, one and two weeks after intervention). The intervention group was treated with folinic acid (70 µg/kg/day) for two weeks. We calculated median concentrations [25th and 75th percentiles].

Results:

Median tHcy concentrations at the three time points did not differ from each other in the control group nor in the intervention group. We also could not observe different tHcy concentrations between both groups. Plasma folate concentrations increased in the intervention group (mean increase 167% (95% CI -291;625)) compared to control group (mean increase -12% (95% CI -132;108)), p for treatment effect:0.03.

Conclusion:

We could not demonstrate a homocysteine lowering effect of folinic acid administration in newborns. This indicates that one carbon metabolism in newborns differs form adults. Cobalamin might be a better strategy to lower tHcy concentrations in newborns.

Clinicaltrials.gov, NCT00877227

Keywords: Cerebrovascular accident, stroke, newborns, homocysteine, folinic acid, folic acid

Introduction

The overall incidence of cerebrovascular accident (CVA) in childhood is very low (approximately 3/100.000 per year)(Raju et al., 2007). In strong contrast, CVA's occurring in the perinatal period have a much higher incidence(Raju et al., 2007). It is estimated that the incidence of ischemic perinatal stroke is 1 in 2300 to 5000 births and accounts for 30% of children affected with cerebral palsy(Raju et al., 2007). Of major importance is the fact that about 10% of newborns admitted to a neonatal intensive care unit suffer from a CVA. Known potential risk factors of the latter are prematurity, asphyxia, hypoglycemia, polycythemia, catheter-related complications, infectious factors, persistent fetal circulation, intra-uterine growth restriction and maternal factors such as diabetes or antiphospholipid syndrome(Raju et al., 2007; Grabowski et al., 2007). Independent of the possible cause CVA can result in significant morbidity, varying from mild hemiplegia to severe cerebral palsy, sensory impairments, mental retardation and even death. Yet aspects of the multifactorial pathophysiology leading to CVA in the newborn remain unclear and in about one third of cases no obvious cause or risk factors can be diagnosed(Akar et al., 2001).

Elevated total homocysteine (tHcy) concentrations have been found to be a risk factor in adults for development of cardiovascular disease and venous and arterial thrombosis including cerebrovascular accident. Elevated tHcy concentrations can, amongst others, be caused by genetic polymorphisms of the methylenetetrahydrofolate reductase gene and/or cystathionine B synthase gene(van der Linden et al., 2006). Non-genetic factors such as folate, vitamin B12 and/or B6 intake, age, sex, estrogen, alcohol consumption and smoking can also influence tHcy(Gellekink et al., 2005; Verhoef et al., 2005; Ueland et al., 1989).

In adults, tHcy can be easily, safely and quickly lowered by 0.2 to 5 mg folic acid each day, even in the absence of elevated tHcy concentrations(den Heijer M. et al., 1998; van Oort et al., 2003; Brattstrom et al., 1988). Current intervention trials show negative results from secondary prevention regarding the cardiovascular risk reduction. Meta-analyses on CVA and elevated tHcy concentrations however suggest that B-vitamin supplementation may

lower the risk of CVA by approximately 20-25%(Herrmann et al., 2007; Homocysteine Lowering Trialists' Collaboration, 2000). Wang et al performed a meta-analysis on folic acid supplementation in relation to stroke and observed an overall decrease of 18%. Furthermore, their results suggested that folic acid supplementation can significantly reduce the risk of stroke in primary prevention (RR 0.75[0.62;0.90] (Wang et al., 2007).

Van Beynum *et al* demonstrated that elevated tHcy concentrations increased the risk of ischemic stroke in Dutch children aged 0-18 years(van Beynum et al., 1999). Sirachainan *et al* found an odds ratio of 8.2 [95% CI 1.4-47.2] for developing ischemic stroke in patients aged ≤ 18 years with elevated tHcy concentrations(Sirachainan et al., 2006). In a previous study, we described significantly higher mean tHcy concentrations in 24 newborn infants with stroke compared to 94 healthy newborn infants (OR 3.95 [95% CI 1.53-10.16](Hogeveen et al., 2002). These studies suggest that elevated tHcy concentrations are also a risk factor for CVA in children and newborns. Since the incidence of CVA in patients admitted to a neonatal intensive care unit is relatively high, we conducted the first prospective randomized folinic acid intervention study in newborns to study whether this risk factor can be modified in this age category.

Subjects and Methods

Subjects

We conducted a prospective randomised intervention study. All newborns admitted to the neonatal intensive care unit in the University Medical Centre Nijmegen, the Netherlands in 2003 were eligible to the study. Those with midline-defects, ECMO-treatment, overt renal failure or blood transfusion were excluded. After written informed consent was obtained, the subjects were randomised for receiving daily folinic acid supplement (intervention group) or no supplement (control group). No supplement was given because a placebo-effect is unlikely in this age category. Parents, nurses and physicians were not blinded to group assignment. Patients received normal medical care during this research project. Evaluations of cerebral ultrasounds and/or scans were performed by physicians not involved in this project and unaware of supplementation. Randomization was centralized but not computerized. This means that for each participant an envelope was drawn by the research assistant at the time of inclusion. These envelopes, filled with “intervention” or “control” notes were mixed thoroughly before they were stored in a box. This study was approved by the local ethics committee (Committee on Ethics in Research with Human Subjects Nijmegen).

Data collection

Clinical data from the newborn were obtained daily from the medical record. We obtained data on gestational age, birth weight, newborn sex, days on ventilator, laboratory results, sepsis/meningitis, intraventricular bleeding or stroke. When newborns were transferred to another hospital during the study period daily folinic acid supplementation (intervention group) was continued and blood samples were drawn as planned.

Blood sampling

Blood was drawn either capillary or arterially during routine blood sampling. Samples were taken within 48 hours after birth (baseline), at day 8 ±1 day (one week) and at day 15 ±1 day (two weeks). EDTA blood (0.5-1.0 ml) was placed on ice immediately and centrifuged within 2 hours. Plasma and cell pellets were separated and stored at -30^{0}C until analysis.

Folinic Acid supplementation

Folinic acid was given for two weeks as 5-formyltetrahydrofolate (10 mg/ml) (Pharmachemie bv, Haarlem, the Netherlands). This solution was administered either intravenously (first week) or orally. To lower tHcy in adults 5 mg/day folic acid is frequently used. Using an average bodyweight of 70 kg for adults we calculated a daily dose of 70 µgram/kg/day for our newborns.

Biochemical analyses

Plasma tHcy was determined in EDTA plasma by an automated high-performance liquid chromatography method with reverse-phase separation and fluorescent detection (Gilson 232-401 sample processor, Spectra Physics 8800 solvent delivery system and Spectra Physics LC 304 fluorometer) as described by Poele-Pothof (te Poele-Pothoff et al., 1995). Plasma folate was determined in EDTA plasma in a competitive immunoassay (Immulite 2000, Siemens Medical Solutions Diagnostics).

Statistical analysis

Results were presented as median values with their 25th and 75th percentiles. Differences between groups were determined by Mann-Whitney test or independent samples T-test where appropriate. Statistical analyses were all performed using SPSS 14.0 for Windows.

Results

We have included 37 newborns in our study (19 intervention group, 18 control group). Characteristics of both groups are presented in Table I. Newborns were admitted because of prematurity, respiratory, infectious or circulatory problems. They were between 26 and 40 weeks of gestational age and between 600 and 3500 grams. Parameters were not significantly different at baseline between the intervention and control group.

Figure 1 shows individual plasma tHcy and folate concentrations at baseline and after one and two weeks from both groups. Due to transfer to another hospital, death, loss of samples and/or too little amount of blood sampled data were not complete for all newborns. In table II median tHcy and folate concentrations at three time points in both groups and mean change between baseline and 2 weeks are shown. Neither plasma tHcy nor folate concentrations were correlated to gestational age, birth weight or creatinine at the three time points. Plasma tHcy concentrations did not differ significantly at the three time points in the intervention group nor the control group. Plasma tHcy concentrations in the intervention and control group were not different. Plasma folate concentrations at two weeks in the intervention group were significantly higher compared to concentrations at two weeks in the control group.

Discussion

In contrast to our expectation we could not demonstrate a homocysteine lowering effect of folinic acid administration in newborns in this prospective randomized intervention study. This unexpected finding might be influenced by folinic acid dose, time of blood sampling, way of administration or other factors. However, it could also indicate that homocysteine metabolism in the neonate may differ from metabolism later in life.

We are fully aware that this is a small study with only 37 patients included. Still, since data on folate and homocysteine in newborns are scarce, we believe that our results add information on one carbon metabolism in this age category. We have performed an intervention study without placebo group because a placebo-effect is unlikely in this age category. We strongly do believe that this did not influence our results since every newborn received routine neonatal intensive care. Furthermore, cerebral ultrasounds and/or scans were judged by physicians who were not aware of this study.

Fuller *et al* routinely supplemented preterm infants with 1 mg folate orally (pteroylglutamic acid) to encourage growth rates in erythroblastotic newborns without gross side effects. This is, on a body-weight basis, around 250 times the recommended amount for adults and around 5-20 times the amount recommended at that time for (premature) infants. Based on results from other studies, they extrapolated that 0.05-0.2 mg/day folate appear safe for preterm infants which is comparable to our dose(Fuller et al., 1992). In adults, folic acid supplementation (0.4-5mg per day) reduces tHcy between 13 and 25% even in the absence of folate deficiency or elevated tHcy concentrations(Homocysteine Lowering Trialists' Collaboration, 2005). In a meta-analysis by the Homocysteine Lowering Trialists' Collaboration the effect of folic acid supplementation was more profound with incremental doses of folic acid, higher pretreatment tHcy concentrations or lower pretreatment folate concentrations(Homocysteine Lowering Trialists' Collaboration, 2005). In the present study, we supplemented the newborns with 0.07mg/kg/day folinic acid which is comparable to 5 mg folic acid a day in adults. We considered this dose to be high enough to expect an effect in

newborns since in adults 0.2 mg folic acid or higher a day decreases tHcy concentrations. Blood samples were obtained at baseline and one and two weeks after birth. Since several authors have described a significant tHcy concentration reduction with folic acid within 14 days in adults we considered this a sufficient period of time to study if folinic acid decreases tHcy concentrations(Brattstrom et al., 1988; Wilcken et al., 1988).

Folic acid supplementation was given as 5-formyltetrahydrofolate (folinic acid), an active form of folate containing a one carbon group for metabolism. Several studies show that folinic acid given either intravenously or orally was as effective or even better than folic acid in lowering plasma tHcy concentrations(Hauser et al., 2001; Buccianti et al., 2001; Yango et al., 2001; Touam et al., 1999). Ducloux *et al* showed a similar tHcy lowering effect of folinic acid administered intravenously compared to orally supplemented folinic acid. They also observed a similar tHcy lowering effect of intravenously administered folinic acid compared to folic acid in adults (Ducloux et al., 2002). Based on these results and the possibility of intravenous administration we considered folinic acid as the best way to study its lowering effect on tHcy in newborns.

Data on the pattern of neonatal tHcy and plasma folate concentrations are scarce. Median tHcy between 6.3-6.8 μmol/L and mean tHcy between 6.1-7.8 μmol/L have been described in umbilical cord blood or the first days after birth(Minet et al., 2000; Karademir et al., 2007; Couto et al., 2007; Guerra-Shinohara et al., 2002; Bjorke Monsen et al., 2003; Refsum et al., 2004; Hongsprabhas et al., 1999). At day 7 Jyothi *et al* describe median tHcy concentrations of 6.3 μmol/L [p25-p75:2.8-8.4 μmol/L] whereas Fokkema *et al* observed mean tHcy concentrations of 6.4±2.6 μmol/L at day 10 and 6.7±2.4 μmol/L at day 20(Jyothi et al., 2007; Fokkema et al., 2002). Our data are comparable with these results. Hongsprabhas *et al* describe an increase in mean tHcy concentrations in the first two weeks of life(Hongsprabhas et al., 1999). In our study, tHcy concentrations do not differ between baseline and one and two weeks after birth neither in the control group nor in the intervention group.

With respect to folate, mean neonatal plasma concentrations of about 25 nmol/L have been described(Minet et al., 2000; Couto et al., 2007; Guerra-Shinohara et al., 2002; Schulpis et al., 2004; Refsum et al., 2004) which is comparable to our results. These relatively high plasma folate concentrations in newborns compared to adults might explain the loss of correlation with tHcy concentrations in this age category.

The past few years, several authors describe a stronger relationship in infants between cobalamin and tHcy than between plasma folate and tHcy(Minet et al., 2000; Bjorke Monsen et al., 2001; Guerra-Shinohara et al., 2002; Molloy et al., 2002; Fokkema et al., 2002). Bjorke-Monsen *et al* describe even an absence of correlation between plasma folate and tHcy (r= -0.06) in 173 apparently healthy newborns at day 4 while correlation between cobalamin and tHcy was high (r=-0.52)(Bjorke Monsen et al., 2001). Minet *et al* also describe a better correlation between cobalamin and tHcy in comparison to plasma folate and tHcy (r=-0.64 and r= 0.30 respectively)(Minet et al., 2000). Van Beynum *et al* suggest that homozygosity for MTHFR 677C->T polymorphism only influences homocysteine concentrations in children (0-19 years) with low folate status(van Beynum et al., 2005). Taken together, cobalamin might be a more important determinant of tHcy than plasma folate in infants and thus cobalamin treatment may be a better choice to decrease tHcy concentrations in infants and newborns (Bjorke-Monsen et al., 2008).

During our study period, four children suffered from a CVA. Although this study was not designed to explore the effect of folinic acid supplementation on the incidence of CVA, only one patient from the intervention group had a CVA compared to three newborns in the control group.

In conclusion: we could not observe a homocysteine lowering effect of folinic acid supplementation in a two weeks intervention study in newborns. This observation was unexpected because similar conditions in adults substantially lower tHcy concentrations. Our findings indicate that one carbon metabolism in newborns differs from adults. Recent data suggest that cobalamin is a stronger determinant of tHcy concentrations in newborns. Thus,

cobalamin supplementation offers an attractive alternative and further studies should focus on cobalamin.

List of abbreviations

CVA	cerebrovascular accident
tHcy	total homocysteine concentrations
NICU	neonatal intensive care unit
CI	confidence interval

There are no conflicts of interest. We do not have to declare any competing interests. There is no funding to report.

Figure 1. Two weeks follow-up of homocysteine and folate concentrations in newborns in the control and intervention group.

Individual data are shown. Concentrations at baseline, after 1 and 2 weeks are depicted.

Figure 1a. Plasma homocysteine concentrations in the control group.

Figure 1b. Plasma homocysteine concentrations in the intervention group.

Figure 1c. Plasma folate concentrations in the control group.

Figure 1d. Plasma folate concentrations in the intervention group.

Table I: Population characteristics.

Data are expressed as median [25th-75th percentile] or number (percentage). CVA = cerebrovascular accident. tHcy baseline=total homocysteine concentration at baseline. Folate concentration=folate concentration at baseline. Independent samples t-test was used to compare groups. P values are calculated using Mann-Whitney U test or Fisher exact test where appropriate.

Table I. Population characteristics of 37 newborns participating in this folinic acid intervention study

	Intervention group	Control group	pvalue
Number of children	19	18	
Gestational age (weeks)	29.7 [28.4;31.3]	29.7 [29.6;34.9]	0.55
Birth weight (grams)	1349 [1108;1640]	1256 [956;1712]	0.63
Male sex, n(%)	9 (47%)	11 (61%)	0.51
Days on ventilator, n [range]	1 [0;2.9]	1.5 [0.5;3.0]	0.90
Sepsis, n(%)	7 (37%)	11 (61%)	0.19
CVA, n(%)	1 (5%)	3 (17%)	0.34
tHcy baseline (µmol/L)	5.6 [3.9;15.1]	7.0 [5.8;13.9]	0.43
Folate baseline (nmol/L)	23 [16;44]	45 [35;53]	0.09

Table II: Changes in homocysteine and folate concentrations in folinic acid supplemented and control newborns during the first two weeks of life.

tHcy=total homocysteine concentration (μmol/L). Fol=folate (nmol/L). Values are expressed as median and 25th and 75th percentile. Intervention consisted of 70 μgram/kg/dag folinic acid intravenously or orally. Differences between intervention and control group are tested using Mann-Whitney test. P value < 0.05 was considered significant.

TABLE II: Changes in homocysteine and folate concentrations in folinic acid supplemented and control newborns during the first two weeks of life

	Median tHcy [p25-p75] baseline	**Median tHcy [p25-p75] 1 week**	**Median tHcy [p25-p75] 2 weeks**	**Mean % change (95%CI)**
placebo	7.0 [5.8;14.1]	5.3 [1.4;20.4]	6.1 [2.2;17.1]	2.6% (-82;87)
intervention	5.6 [4.0;16.2]	7.3 [1.9;26.8]	6.3 [2.7,11.9]	111% (-373;597)
p value	0.43	0.33	0.79	0.62
	Median fol [p25-p75] baseline	**Median fol [p25-p75] 1 week**	**Median fol [p25-p75] 2 weeks**	**Mean % change (95%CI)**
placebo	46 [36;53]	31 [15;61]	40 [12;134]	-12% (-132;108)
intervention	23 [16;44]	35 [13;97]	78 [33;184]	167% (-291;625)
p value	0.09	0.44	0.01	0.03

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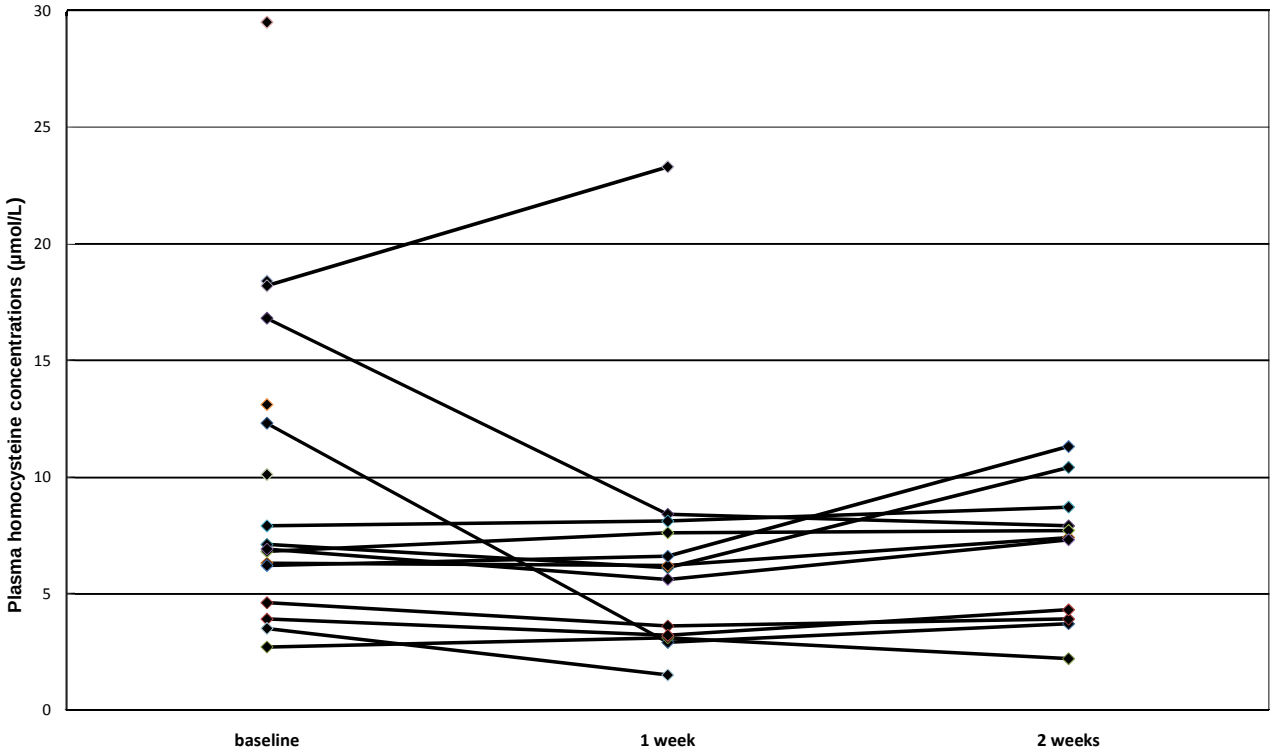
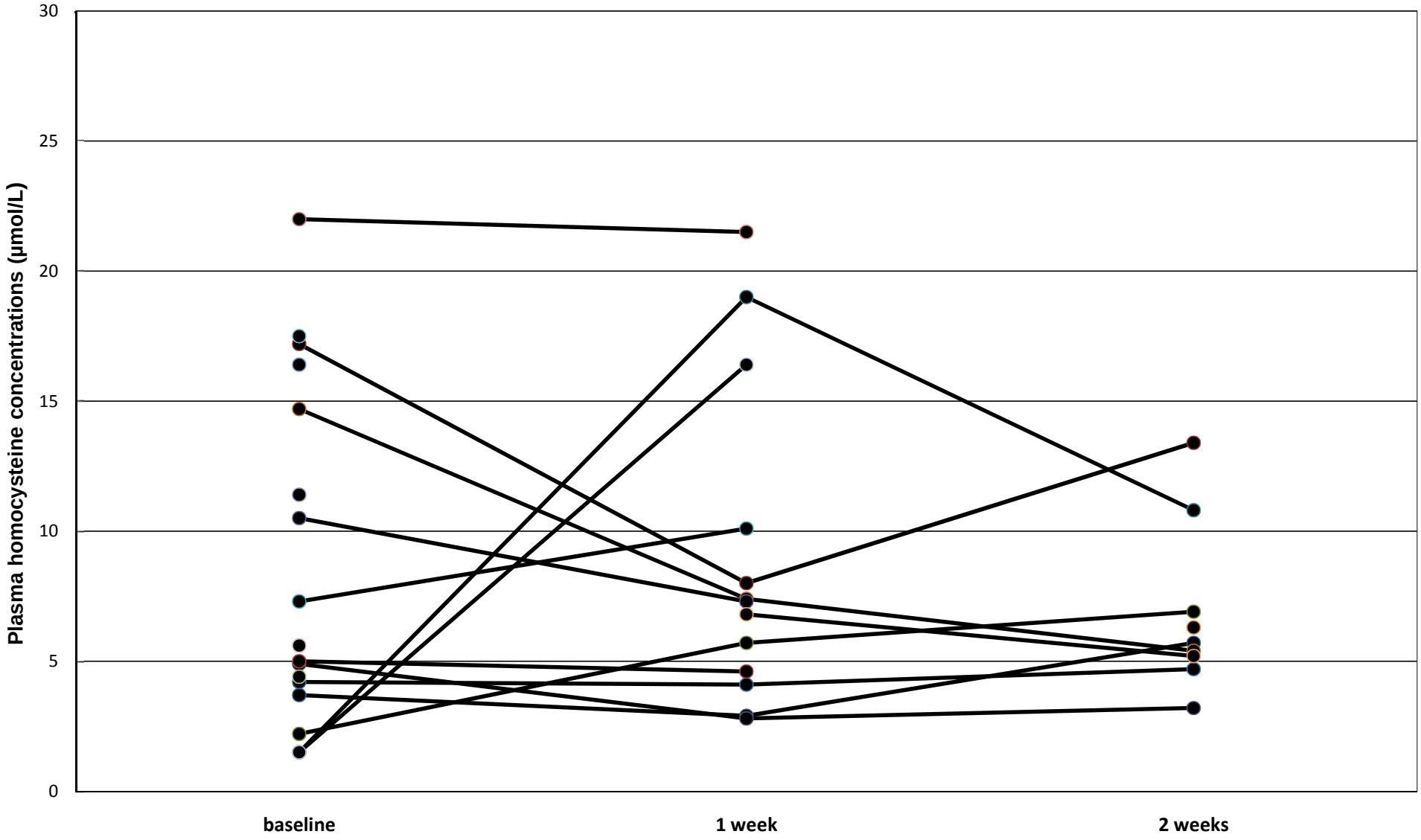
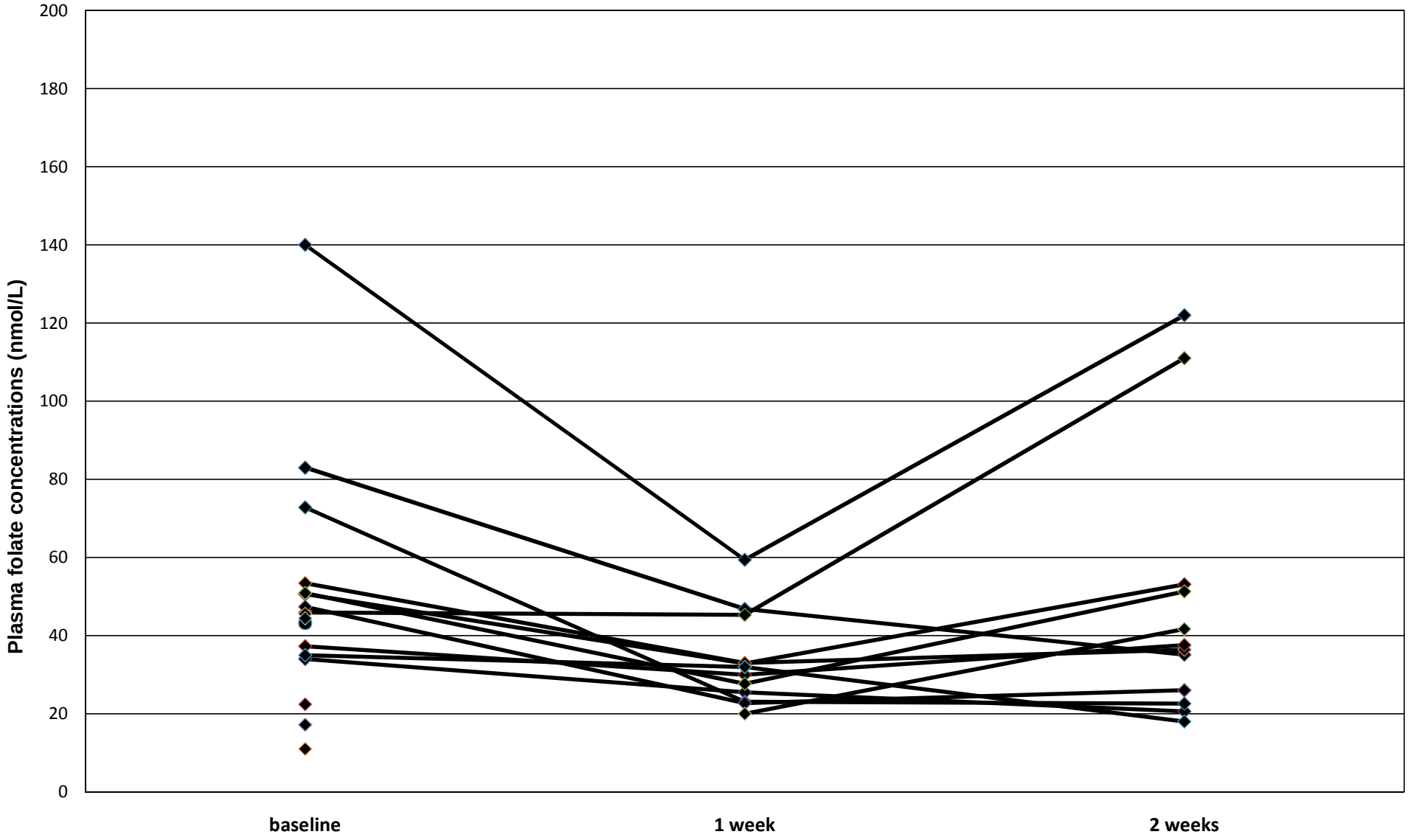
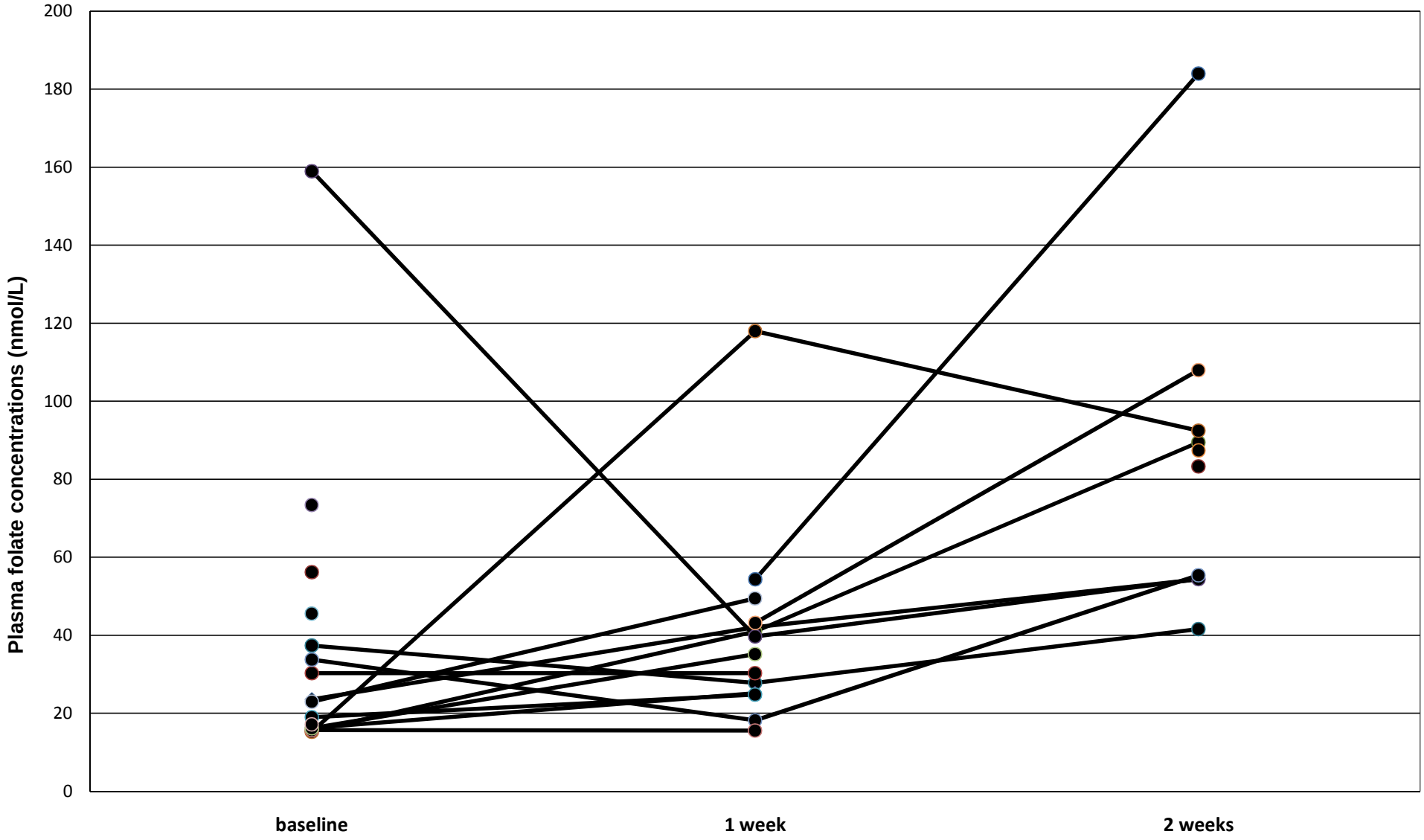

Plasma homocysteine concentrations (µmol/L)
30
25
20
15
10
5
0
baseline
1 week
2 weeks


Plasma homocysteine concentrations (µmol/L)
30
25
20
15
10
5
0
baseline
1 week
2 weeks


Plasma folate concentrations (nmol/L)
200
180
160
140
120
100
80
60
40
20
0
baseline
1 week
2 weeks


Plasma folate concentrations (nmol/L)
200
180
160
140
120
100
80
60
40
20
0
baseline
1 week
2 weeks