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High serum vitamin B12 levels in neurodevelopmental disorders across age groups.
A comparison with healthy controls and schizophrenia

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ABSTRACT

Vitamin B12 deficiency is known to have detrimental effects on neurodevelopment and cognition. Recent studies also suggest that high levels may have negative health implications, i.e. during pregnancy it associates with risk of neurodevelopmental disorders (ND). We therefore examined serum vitamin B12 in children and adults with ND and compared with healthy controls (HC) and schizophrenia, a severe mental disorder with neurodevelopmental abnormalities. Vitamin B12 was measured in patients with ND (n=222), comprising Autism Spectrum Disorders, specific NDs and Intellectual Disability (age range 3 - 53 years), in schizophrenia (n=401), and in HC (n=483). Age-and gender-adjusted vitamin B12 z-scores were calculated by comparisons with a reference population of primary care patients (n=76,148). We found higher mean vitamin B12 level in ND (478 pmol/l, z-score = 0.30) than HC (341 pmol/l, z= 0.06, p<0.01) and schizophrenia (335 pmol/l, z= -0.02, p<0.001). Vitamin B12 remained significantly higher in ND after control for possible confounders as vitamin supplement, hemoglobin, folate, creatinine, alanine aminotransferase, and leukocyte count (p<0.02). Abnormally high vitamin B12 (> 2 SD from mean in reference population) were more frequent in ND than in HC (OR: 4.1, p=0.002) and schizophrenia (OR: 2.5, p=0.03), while abnormally low B12 were equally frequent in ND as in schizophrenia (OR: 0.95, p=0.95) and non-significantly more frequent than in HC (OR: 3.7, p=0.07). To conclude**,** vitamin B12 levels are higher in ND than in HC and schizophrenia across age groups suggesting it may be specific for ND. The result warrants further studies to investigate possible mechanisms.

Introduction

Neurodevelopmental disorders (ND), including Intellectual Disabilities and Autism Spectrum Disorder (ASD) affect at least 2% of the population worldwide, with large suffering for the individual and high mortality [1]. The disease mechanisms underlying ASD and other ND are still largely unknown, but it is well established that both genetic and environmental factors are important. The heritability of ASD is high, and was recently estimated to be 83%, while non-shared environmental factors contribute 17% [2]. According to a new genome-wide association study, variants in *CUBN*, a gene encoding a vitamin B12 receptor, confers risk of major psychiatric disorders including ASD[3]. Of environmental factors, it has been established that advanced parental age, birth complications such as hypoxia, vitamin D deficiency and exposure to heavy metals increase the risk of ASD[4]. The risk factors are though to exert their effect through influencing pathways that are disturbed in autism, such as oxidative stress and inflammation[4].

It is well known that vitamin B12 deficiency during pregnancy and throughout childhood disturbs neurodevelopment [5]. Adequate vitamin B12 status is also necessary for neurocognitive function in adults [6]. Although the exact mechanism for how vitamin B12 influence neurodevelopment is unknown, it's role in oxidative stress [7] and inflammation[8] is intriguing [4]. Vitamin B12 is present in animal products and deficiencies of vitamin B12 is frequent in developing countries [9], where studies have shown low levels of vitamin B12 in ASD children [10,11]. In western welfare societies were diets are rich in animal products, vitamin B12 deficiency is rare [12,13]. Furthermore, the prevalence of ASD has raised markedly in developing countries in parallel with increasing intake of animal products containing vitamin B12 [14]. This makes it unlikely that dietary vitamin B12 deficiency is a cause of the high prevalence rates of ASD. However, patients with ASD are often picky eaters [15], and their

individual diet may still contribute to e.g. vitamin D deficiency [4] and low hemoglobin levels [16]. Two studies have described vitamin B12 in ASD patients from western countries [17,18]. None of these studies found low levels of vitamin B12 [17,18]. Moreover, it was recently reported from USA that high serum levels of vitamin B12 during pregnancy increased the risk of ASD in the offspring [19]. Serum levels of vitamin B12 above the 90th percentile more than doubled the risk of having a child with ASD [19]. Recent clinical studies also suggest that high levels of vitamin B12 associates with neurological diseases, cancer, liver disease and kidney failure [20-22].

However, negative consequences of high vitamin B12 levels have traditionally received little attention. Importantly, new studies have found that increased intake of vitamin B12 results in only minor changes in excretion [23], indicating that vitamin B12 levels do not just reflect on the intake. This is in line with recent findings that levels of vitamin B12 are indeed more influenced by genetic factors, than by environmental factors [24]. Several genetic variants have been found to influence serum levels of B12, most important of them are genes that encode uptake receptors for vitamin B12 (*CUBN*) [25] and proteins that binds vitamin B12 in serum, *TCN1* (passive fraction) and *TCN2* (active fraction) [26,27].

Hereditary vitamin B12 deficiency may lead to diverse disorders ranging from intellectual disability, neurological diseases to mental disorders, and their onset may range from the neonatal period to adolescence [28]. Thus, patients with disturbed vitamin B12 metabolism may present with a wide range of NDs, not only ASD, and when investigating the role of vitamin B12 in neurodevelopment, it is relevant to include patients with a broad range of neurodevelopmental disorders. Neurodevelopmental abnormalities are also a characteristic of schizophrenia, a disorder that shares some clinical features with autism [29,30].

However, to the best of our knowledge, no studies have investigated whether vitamin B12 levels differ among ND diagnostic categories and from schizophrenia.

In the present study, we investigated levels of vitamin B12 in a large sample of patients with ND, using healthy controls and schizophrenia patients as comparison. To adjust for differences in age and gender we also used data from a large reference population of primary health care patients. We investigated the hypothesis that in a western society, abnormally high levels of vitamin B12 would be more common in ND than controls, and that mean serum levels of vitamin B12 would be higher in patients with ND than in controls. Secondly, we hypothesized that vitamin B12 levels would be equal in different diagnostic groups of ND, and equal as in patients with schizophrenia.

Methods

Participants: A total of 1115 participants were included, comprising ND, schizophrenia patients and healthy controls.

Neurodevelopmental disorders (*ND):* Children and adults with ND were included from out-patient clinics for patients with ND in different regions of Norway (Oslo, Akershus, Trondheim and Vestre Viken) from 2012 to 2016. Patients who had been clinically diagnosed with ND were offered to participate in the ongoing BUPGEN study[31] which is based on data from medical records gathered during clinical work-up, with an aim to investigate etiological factors of ND. Included in the current analyses are participants who had blood test results for vitamin B12. The sample comprised 222 patients with ND. Of these n=162 were diagnosed with Autism Spectrum Disorder (ASD) (n=28 with Infantile autism, n=62 with Asperger's syndrome, n=32 with Atypical autism, n=40 with Pervasive DD NOS), and n=62 were diagnosed with other ND (n=16 with Intellectual disability without ASD, n=18 with

Language Disorder and n=27 with mixed delays of specific abilities or ND NOS). As seen in Table 3, there were 72 children under 12 years, 77 were adolescents (12-18) and 72 were adults above 19 years. Patients were mainly recruited from Oslo University Hospital (n=138), St.Olavs Hospital (n=65), Vestre Viken Hospital (n=12) and Ahus Hospital (n=7).

Schizophrenia spectrum disorder: Patients from the Oslo area, who were older than 17 years and registered in the psychiatric services of Oslo University Hospital and collaborating hospitals with a schizophrenia spectrum disorder were asked to participate in the ongoing Thematically Organized Psychosis (TOP) Study in Norway. A total of 401 patients were included in the present sub-study of TOP, from 2003 to 2016, comprising SCID-I verified diagnosis of Schizophrenia (n=313), Schizophreniform (n=27) and Schizoaffective disorder (n=61). There were 14 adolescents and 387 adults.

Healthy controls: A total of n=492 healthy persons of whom 70 were adolescents, and 422 were adults. They were living in the Oslo area, were randomly selected from statistical records, and were invited by posted letters to participate. Exclusion criteria were a history of severe medical or psychiatric diagnoses, including alcohol or illicit substance abuse, or severe mental disorders in first degree relatives.

Reference population: Serum vitamin B12 measurements were sampled from all general practitioners in the Vest-Agder County, southern Norway, which according to Norwegian Institute of Public Health, has approximately equal life expectancy as the Oslo area. The reference group comprised 76,148 anonymous blood test results from 6282 children (3-11 years), 8322 adolescents (12-17 years) and 61544 adults (18-53 years)[32]. As seen in

Supplementary Figure 1, males and females in the reference population had slightly different distributions of vitamin B12. The distribution of vitamin B12 levels differed with age; higher levels and larger variation were found in children than in adolescents and adults. The reference population was used to calculate age- and gender- adjusted z-score for vitamin B12 levels in patients and healthy controls.

Consent: Written informed consent was obtained from all participants in the diagnostic groups and healthy controls (n=1115). The calculations of age- and gender-adjusted z-scores were based on anonymous data from the reference sample [32]. The study was approved by the Regional Ethics Committee and the Norwegian Data Inspectorate.

Study design: The study is a cross-sectional comparison of vitamin B12 serum levels between the diagnostic groups of ND, schizophrenia and healthy controls, with a reference population used for calculation of age- and gender-adjusted standardized z-scores.

Vitamin B12 measurement: Total serum vitamin B12 was measured by standard immunometric methods (Modular E, Roche for most patients, except for 19 patients that had been measured by Dimension Vista, Siemens or Beckman UniCel). The laboratories participate in a regular quality program securing reliable and similar measurements. The B12 reference range used at Oslo University Hospital is 150 - 650 pmol/l, which corresponds well with the data from our healthy adult controls as well as the reference population. Blood levels of hemoglobin and leucocytes, in addition to plasma levels of folate, creatinine, and alanine aminotransferase (ALAT) were measured by standard laboratory methods.

Statistical analysis:

Software: Analysis was performed using IBM SPSS Statistics v. 22 (IBM Corp. 2014). The level of significance was preset to $p < 0.05$ (two-tailed).

Z-score calculations based on reference sample: The total number of blood sample measurements of vitamin B12 from primary health care patients in Vest-Agder county (n=76,148) was used to calculate z-scores for patients and healthy controls. Age-and gender-adjusted z-scores were calculated as earlier described[33]. Briefly, the distributions of continuous variables were evaluated by Q-Q plots and Box-Cox transformed to achieve approximate normality. Then, every participant's vitamin B12 level (pmol/l) was compared with their age- and gender-matched group in the reference population, and a standardized z-score was calculated based on the mean and standard deviation of the age- and gender-matched group of the reference population.

Statistical methods for group comparisons and control of confounders*:*

Whole group comparisons of mean vitamin B12 levels: Comparisons of age-and gender-adjusted z-scores was done by using ANOVA with a post hoc Bonferroni test. Comparisons of raw vitamin B12 levels (pmol/l) was done by doing ANCOVA with control for age and gender, with post hoc Bonferroni test.

Adjustment for age-and gender- differences: We took into account that vitamin B12 levels are influenced by age and gender, and analyzed vitamin B12 by using z-scores that had been age-and gender-adjusted based on comparisons with a reference population.

In addition as we lacked healthy children in our group of healthy controls we performed a sub-analysis among participants excluding children. We included ND and healthy controls above 12 years, and vitamin B12 levels (pmol/l) were compared by using ANCOVA. We adjusted for age and gender by entering gender and diagnostic group as fixed factors, and age as a continuous covariate, and performed a Bonferroni post-hoc test.
To analyze differences in vitamin B12 levels in participants who used vitamin supplements, we ANOVA, and controlled for folate levels by using ANCOVA.

Control for additional possible confounders: We analyzed how vitamin B12 correlated with other blood test results by Pearson's r. In the whole sample we controlled for possible confounders by doing linear regression analyses. Vitamin B12 was set as the dependent variable, while possible confounders as indications of nutritional status (hemoglobin and folate), differences in age, gender, liver function, kidney function, and leukocyte count were entered as independent variables, together with diagnostic group (ND versus healthy controls). In a subsample, we also added control for intake of vitamin B-containing supplements, also by using linear regression analyses.

Comparisons of proportions with abnormal vitamin B12 levels: Abnormal vitamin B12 levels were defined as having an age- and gender –adjusted vitamin B12 z-score more than two standard deviations off of the mean in the age- and gender- matched group from the reference population. Group comparisons of proportions with abnormal levels (z-score) were made by calculating odds ratios.

Results

Descriptives are shown in Table 1. As seen mean age and gender differed among the diagnostic groups; patients with ND were younger, and more frequently male, and their intake of vitamin supplement was equal as in healthy controls and schizophrenia. Levels of folate was 20 pmol/l which was higher than in healthy controls (18 pmol/l p= 0,02) and higher than in schizophrenia (16 pmol/l, $p<10^{-7}$). They also had higher leukocyte count than healthy controls ($p<0.001$), and lower hemoglobin and creatinine ($p<0.001$).

Mean serum level of vitamin B12 in ND compared with healthy controls and schizophrenia: In ND the mean serum vitamin B12 was 478 pmol/l, corresponding to a mean z-score of 0.30, which was significantly higher than in healthy controls (341 pmol/l, z-score=0.06, p=0.01) and higher than in schizophrenia (mean 335 pmol/l, z= -0.02, p=0.0004), as shown in Table 2 and Figure 2.

In Table 3 we present mean levels of vitamin B12 in different diagnostic entities of ND. ANOVA analysis found no significant differences in mean vitamin B12 serum levels among the different ND diagnostic entities (p=0.08).

Control for possible confounding factors:

Age group: Mean vitamin B12 z-scores in different age groups of ND were not significantly different (children z=0.22, adolescents, z=0.27, and adults z=0.41, p=0.57) and ANCOVA showed no significant interaction effect between age groups and diagnostic groups on levels of vitamin B12 (p=0.50). A statistical sub-analysis including only participants older than 12 years old were performed in order to compare ND with an age-matched group of healthy controls. We found that when excluding children, ND had higher vitamin B12 than

healthy controls (403 pmol/l vs. 340 pmol/l, Cohens *d* 0.44), which was a highly significant difference ($p<1x10^{-6}$) after control for age and gender.

Supplement and nutritional differences: As seen in Table 1, folate were lower in ND than in SCH, and higher in ND than in healthy controls. A possible reason could be differences in nutrition and supplement intake. We had information regarding intake of vitamin B12 supplement for a subsample consisting of 120 participants. In this subgroup, all participants were from the Oslo area, and had been analyzed in the same laboratory, their mean age was 29 years, with no significant differences between groups regarding age, gender, levels of folic acid, hemoglobin, liver or kidney function. As seen in table1, intake of vitamin B12-containing supplement was equally frequent across groups, 22 % in ND and Schizophrenia, and 28% in healthy controls (p=0.70). Participants who had taken vitamin supplement had significantly higher levels of vitamin B12 (505 pmol/l, z=0.0) than those who had not (355 pmol/; z=0.80, p=0.002). In these participants from the Oslo area, levels of vitamin B12 was also higher in ND than in healthy controls after controlling for intake of vitamin B12 supplement, age and gender (p<0.0003). Although participants who had taken vitamin supplements had significantly higher levels than those who had not (p=0.002), this difference was no longer significant when we controlled for levels of folate (p=0.14), supporting that to controlling for folate could serve as a proxy for intake of vitamin B12-containing supplement. In the ND group, the portion of children was high, and as seen in table 1, and as expected, they had lower hemoglobin [34] and creatinine [35] than the other diagnostic groups. They also had higher leukocyte count, as a sign of increased inflammation. We therefore controlled for these possible confounders by conducting a linear regression analysis, with vitamin B12 as the dependent variable and the possible confounders (age, gender, folate, hemoglobin, markers of kidney function, liver function and

inflammation) as independents together with diagnostic group (ND or healthy controls). ND had higher mean serum vitamin B12 (z-scores), after control for differences in folate, hemoglobin, ALAT, creatinine, and leukocyte count (p=0.02).

Comparisons with control for confounders using the raw values of vitamin B12 in pmol/l was performed In a sub-analysis within participants older than 12 years old, which gave similar results as the calculation with z-scores, meaning that serum vitamin B12 (pmol/l) were higher in adults and adolescents with ND after control of age, gender, folate, hemoglobin, ALAT, creatinine and leukocyte count (p=0.0004). And in a subanalysis shown in table 4, we also added control for confounders by analyzing subsample from the Oslo area, and found that vitamin B12 levels was still higher in ND than in healthy controls (p= 0.001) after control for age, gender, vitamin supplement and other blood test results.

Proportions with abnormal vitamin B12 levels in ND, schizophrenia and healthy controls: Participants with *high* vitamin B12 (defined as a z-score more than two standard deviations above the mean in the reference population) had a mean vitamin B12 level of 856 pmol/l (range 641-1475 pmol/l) and this high level was present in 6.3 % of patients with ND, versus in 1.6 % of healthy controls, corresponding to an odds ratio of 4.1 (p=0.002). Among patients with schizophrenia, 2.7 % had a high serum level of vitamin B12 (z-score > 2) versus 6.3 % in ND, corresponding to an odds ratio of 2.5 (p=0.03). Participants with *low* vitamin B12 (defined as z-score < 2) had a mean vitamin B12 level of 148 pmol/l (range 99-294 pmol/l) and this low level was present in 2.3% of patients with ND, versus 0.6% of healthy controls corresponding to an odds ratio: 3.8 (p=0.07). Among patients with schizophrenia, 2.2% had a

low vitamin B12 (z-score < 2), corresponding to an equal odds ratio as in ND (OR: 0.95, p=0.95).

Discussion

The main finding of the present study is that patients with ND have significantly higher serum level of vitamin B12 than healthy controls and patients with schizophrenia across age groups. Having a high vitamin B12 level was 4.1 times more frequent in patients with ND than in healthy controls, while having a low vitamin B12 level was not significantly more frequent (OR 3.8, p=0.07). These findings obtained from the largest study of levels of vitamin B12 in ND patients to date could imply vitamin B12 related pathological mechanisms are of importance for ND in adolescence and adulthood.

Our results seems in line with a recent study reporting that high levels of vitamin B12 during pregnancy was associated with doubled risk of ASD in the child, and that both high and low intake of vitamin B12-supplements was associated with increased risk[19]. Our result is also supported by a study that found that 18% of patients with ASD had levels above a common current reference range[17], and findings of a non-significant trend of higher vitamin B12 levels in ASD [36]. Furthermore, it seems supported by a study reporting higher levels in patients with neurological diseases.[22]

Previous studies of vitamin B12 in ASD used a case-control study design, which makes it difficult to evaluate if the differences regarding vitamin B12 are specific for ASD versus other ND. In the present study we found no significant differences between vitamin B12 and diagnostic categories of ND. Vitamin B12 deficiency may not only cause ND, but may also cause psychotic symptoms [37] as in schizophrenia, a severe mental disorder characterized by

neurodevelopmental abnormalities that shares some clinical characteristics with ASD [29,30]. Vitamin levels tend to be equal in groups of similar socioeconomic status [38], and schizophrenia patients are, like ASD, characterized by social deficits and by increased needs for social disability benefits, community housing, and medication. Even so, schizophrenia patients do have adequate vitamin B12 levels according to a meta-analysis [39], as in our study. Our result of high vitamin B12 levels in ND while not in schizophrenia suggests that underlying mechanisms are not related to sociodemographic factors, but rather to disease specific pathology of ND.

The current vitamin B12 laboratory reference limits for healthy persons are usually equal across age groups [21,40], although, several studies have indicated that vitamin B12 levels might be higher in children than in adolescents and adults [12,41]. Therefore we controlled for age- and gender-differences in vitamin B12 by transforming the raw serum concentration (pmol/l) into age-and gender-adjusted z-scores based on a large reference population. In our reference population, young children from primary health care had larger variation in vitamin B12 levels than adults. As pediatric reference levels for children are still uncertain, and as we lacked healthy children as controls, we performed a sub-analysis where children were excluded, using the raw B12-concentrations in pmol/l, comparing ND with an age-matched group of healthy controls, and adjusting for age and gender. This sub-analysis found similar results of higher levels of vitamin B12 in ND than in healthy controls with strong statistical significance ($p<1x10-6$). Levels of vitamin B12 were higher in participants who had taken vitamin B12-containing supplements, and B12 levels were positively with folate, liver enzymes and leukocyte count, and were negatively correlated with creatinine and hemoglobin. Our results were therefore adjusted for these possible confounders and patients with ND still had significantly higher vitamin B12 than healthy controls.

Possible Pathological Mechanisms: The mechanisms behind high vitamin B12 levels are unknown, but there are some indications of potential explanations from the literature. First, in clinical settings, high levels of vitamin B12 associates with high *TCN1*[22], the major transport molecule for vitamin B12 in serum [27,42]. Genetic variants in TCN1 influence vitamin B12 levels [24] and high gene expression of TCN1 was recently found to associate with poor verbal memory [43], a cognitive impairment which may be relevant for patients with ND.. Also, patients may have higher vitamin B12 levels due to variants in *CUBN*, a gene important for uptake and excretion of vitamin B12[25,44] and a risk factor for psychiatric disorders and ND [3].

Second, immune related pathology may be an underlying mechanism, as this associates with ND [4]. Autoantibodies (immunoglobulins) may react with vitamin B12, and the antibody-binding may reduce the kidney excretion and the cellular uptake, and lead to higher serum levels. Previous studies suggest that 8-25 % of patients with elevated vitamin B12 have antibodies against vitamin B12 [45]. Also, autoantibodies against the folate receptor may be involved. Such antibodies have been found in increased amounts in ASD [46-50], and have been associated with high vitamin B12 [51].

Third, the high vitamin B12 levels may be linked to oxidative stress, an abnormality of relevance for ASD [4] and vitamin B12 [7]. High vitamin B12 associates with a marker of oxidative stress in cerebrospinal fluid [8] and hypoxia, which is a risk factor for ASD [4], increase oxidative stress and elevate vitamin B12 [52]. In line with this, patients with bronchopulmonary disease, a condition with frequent hypoxia, was found to have high levels of vitamin B12[22].

Fourth, possible mechanisms could involve deficient intracellular utilization of vitamin B12 [53] as high serum levels have been observed in patients that lacked the active transcobalamin bound vitamin B12[22], lacked active methylcobalamin[54] and lacked ability to intracellular utilization due to a hereditary vitamin B12 disease [55]. Reduced cellular utilization could possibly be caused by increased expression of *TCN1*, which binds vitamin B12 in serum and renders it unavailable for cellular uptake[26,53].

Limitations/strengths: A strength of this study is that it is the largest study of vitamin B12 in ND so far. It is also the first study to compare levels in ND with levels in schizophrenia, a disorder that shares both genetic risk factors and sociodemographic factors. Another strength is that our study includes adults, where reference limits for vitamin B12 are more established than for children and adolescents. The findings of normal vitamin B12 levels in schizophrenia are in line with previous meta-analyses, and the vitamin B12 level in our healthy controls are similar to a another recent study from Norway [13], and as in the reference population of 76,148 blood samples, which supports that our comparison groups are representative.

Our control for confounders is also a strength. We adjusted for ALAT and creatinine, as kidney failure and liver disease may increase vitamin B12 levels. We also controlled for differences in leukocyte counts, as immune abnormalities are associated with ASD [4] . A limitation in the current study is lack of information regarding vitamin supplements in all participants. Importantly, vitamin B12 levels have been found to be influenced mostly by genetic factors and by non-shared environmental factors[24], suggesting that factors other than diet may be major determinants. In the subgroup that we had information regarding vitamin intake, the percentage was equal in ND as in schizophrenia and healthy controls. Furthermore, in our study as in others, vitamin B12 levels associate with levels of folate[24],

and in Norway, the vitamin B12 supplements that are commonly available, contain both vitamin B12 and folate. In line with this, our analyses showed that vitamin B12 levels was not significantly associated with supplement intake after controlling for folate. Therefore we controlled for folate as a proxy for vitamin supplement in the whole sample, and this did not change the main result. Furthermore, in a subgroup we controlled for both supplement intake, folate, hemoglobin levels, (as an indicator of general nutritional status) and other possible confounders, and vitamin B12 was still significantly higher in ND than in healthy controls.

Clinical implications: The current results suggest that high vitamin B12 may be of clinical relevance in ND. Increased mortality has been reported in ND [1], and high vitamin B12 has been associated with neurological disease, bronchopulmonary disease and other severe somatic conditions [20-22]. Thus, high vitamin B12 levels in patients with ND could be regarded as a possible sign of underlying disease.

Conclusion: Children and adults with ND have higher serum levels of vitamin B12 than healthy controls and schizophrenia patients. High vitamin B12 levels seem to have clinical relevance as a predictor of severe outcome in many diseases. Further research is needed to identify possible mechanisms.

Contributions:

S. Hope, T.Nærland and O.A. Andreassen conceived the study and its design, S. Hope and L. Mørkrid performed statistical analysis, S. Hope, A.L. Høiland, T Torske, E. Malt, M. Nerhus, K. Wedervang–Resell, N. E. Steen, I. Agartz, N. Stenberg J. Johannessen T.G.Abrahamsen and T. Hundhausen contributed to data acquisition and interpretation of results, S. Hope and T. Nærland wrote the manuscript, which was revised for intellectual content by all other authors. All authors approved the final version of the manuscript.

Abbreviations:

ND: Neurodevelopmental disorders

ASD: Autism Spectrum Disorders

Figure Legends:

Mean serum levels of vitamin B12 in children, adolescents and adults in Neurodevelopmental disorders, Schizophrenia, Healthy controls and in a reference population of primary health care patients from southern Norway. Serum levels are shown as age-and gender adjusted z-scores.

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