Cerebrospinal Fluid Monoamines in Prader–Willi Syndrome

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Background: *The behavioral phenotype of Prader–Willi syndrome (PWS) suggests hypothalamic dysfunction and altered neurotransmitter regulation. The purpose of this study was to examine whether there was any difference in the concentrations of monoamine metabolites in the cerebrospinal fluid (CSF) in PWS and non-PWS comparison cases.*

Methods: *The concentration of monoamine metabolites in CSF was determined in 13 children and adolescents with PWS diagnosed on clinical and genetic criteria. The concentrations were compared with those from 56 comparison cases in healthy and other contrast groups.*

Results: *The concentrations of dopamine and particularly serotonin metabolites were increased in the PWS group. The differences were most prominent for 5-hydroxyindoleacetic acid. The increased concentrations were found in all PWS cases independently of age, body mass index, and level of mental retardation.*

Conclusions: *The findings implicate dysfunction of the serotonergic system and possibly also of the dopamine system in PWS individuals, and might help inform future psychopharmacologic studies.* Biol Psychiatry 1998;44: 1321–1328

Key Words: Prader–Willi syndrome, behavior, cerebrospinal fluid, neurochemistry, serotonin, dopamine

Introduction

The Prader–Willi syndrome (PWS) is characterized by perinatal muscular hypotonia and infancy feeding problems, followed by overeating and excessive overweight, usually before age 2–4 years, as well as other symptoms related to hypofunction of the hypothalamus. Learning disorder, hypogonadism, short stature, small hands and feet, hypopigmentation, and a typical appearance are other characteristic features (Prader et al 1956; Holm 1981; Cassidy 1984; Greenswag 1987; Butler 1989; Butler and Meaney 1991). PWS patients also have a typical behavior pattern (Whitman and Acardo 1987; Curfs et al 1991). Children with PWS are generally more sleepy and slow moving during the daytime, and show lots of rituals and compulsive behavior, particularly skin picking, causing wounds on hands, arms, legs, and face (Clarke 1988; Greenswag 1987; Cassidy et al 1989; Dykens 1996). Up to 4–5 years of age they are often rather docile, but as obesity increases they become more belligerent, throw temper tantrums, and from about 5–7 years of age, the skin picking begins (Åkefeldt 1992).

The incidence of the syndrome is estimated at 1 in 8000–10,000 newborns in North Dakota, and in Skaraborg, Sweden (Burd et al 1990; Åkefeldt et al 1991). In most cases PWS is associated with damage on the proximal portion of the long arm of chromosome 15 (Ledbetter et al 1981). The deletion can be found by molecular genetics on chromosomes inherited from the father (Nicholls et al 1989; Knoll et al 1993a). Genomic imprinting, due to variations in the methylation pattern between the paternal and maternal allele can be detected (Swain et al 1987; Nicholls et al 1989; Driscoll et al 1992; Barlow 1993) in some cases. Different variants of numerical imbalance between the materials inherited from the two parents are seen. Maternal dominance, for example maternal disomy (Nicholls et al 1989; Mascari et al 1992; Knoll et al 1993b), is the rule. Other reasons for loss of genetic material or imbalance, such as translocation (Ledbetter et al 1982; Wenger et al 1989) or a marker chromosome 15 (Wisniewski et al 1980; Baker et al 1994) have been found.

Brain monoamines have been shown to be connected with eating behavior. Several studies have given support to the hypothesis that serotonin plays an important role in the regulation of satiety (for review see Leibowitz 1986, 1990). Serotonergic dysregulation is believed to play a role in the pathophysiology of anorexia nervosa. In a controlled study by Kaye et al (1991), female underweight anorectic subjects had low levels of 5-hydroxyindoleacetic acid (5-HIAA), the main metabolite of serotonin, in the cerebrospinal fluid (CSF); normal levels in short-term weight-restored, and elevated levels in long-term weight-

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Received September 24, 1996; revised June 13, 1997; revised October 16, 1997; accepted October 30, 1997.

0006-3223/98/$19.00
PII S0006-3223(97)00519-2

restored subjects were found. Clinical improvement in bulimic patients might be achieved with tricyclic antidepressants that act in part on serotonin (Geracioti and Liddle 1988).

The Purpose of the Present Study

The specific behavior of PWS patients and the suspected connection with hypothalamic dysfunction indicates a disturbed neurotransmitter regulation in these patients. In the present study we have determined the serotonin metabolite 5-HIAA, the dopamine metabolite homovanillic acid (HVA), and the noradrenaline metabolite 4-hydroxy-3-methoxyphenyl-ethylene glycol (HMPG) in the CSF of PWS patients and comparison groups, with the aim of elucidating the PWS pathophysiological mechanisms and possibly finding a means to monitor efficacy of therapy.

Methods and Materials

PWS Patients

Thirteen individuals with PWS, 8 male and 5 female, diagnosed according to the consensus diagnostic criteria (Holm et al 1993), had the characteristic methylation pattern detected by D15S63 (PW71), visualized with molecular genetics (Dittrich et al 1992; Reis et al 1994). They constituted the "PWS" group. They were all mildly mentally retarded (IQ 50–70). The mean age was 8.0 years (range 0.4–23 years). Lymphocyte DNA was isolated and analyzed with polymorphic DNA markers from the chromosomal region 15q11–13. In all cases the microdissected probe D15S63 (PW71) was used, and in most of them the D15S9, D15S10, D15S12, D15S13, and GABRB3 also (Åkefeldt et al 1995).

Comparison Groups

Five groups were included for contrast.

HEALTHY GROUP (NO. 1). This group included 15 individuals, 11 male and 4 female. Six of these were "younger" (mean age 5.8 years, range 3.6–8.8 years). They were selected from children who had presented with slight to moderate symptoms of headache or other soft signs of a possible neurologic character at a pediatric clinic; however, careful medical and laboratory examination revealed no sign of central nervous system (CNS) disorder. The lumbar puncture was performed for diagnostic purposes to exclude infections (usually Borrelia). Normal values for albumin, immunoglobulin (Ig)G, and IgM were found in the cerebrospinal fluid, and no oligoclonal bands occurred, which excludes inflammatory processes.

Nine of the healthy group were "older" (mean age 20.9 years, range 18–25 years). They were recruited from healthy volunteers, consecutively examined as participants in a larger study on CSF biochemistry in healthy individuals at our department (Blennow et al 1993b). The mean age in the whole healthy group No. 1 was 14.8 years (range 3–25 years).

HEALTHY GROUP (NO. 2). The second contrast group was included in another study from our department (Gillberg et al 1983). This group comprised 22 children, 16 boys and 6 girls. The mean age was 9.4 years (range 3–14 years). The children were judged "normal" and none of them were on pharmacologic treatment at the time of the lumbar puncture. The criteria for "normality" for this group was 1) normal intelligence, 2) no known neurological handicap, and 3) no medication at time of lumbar puncture. All the children in this comparison group had had their CSF examined because of some clinical suspicion of neurological disorder. During the investigations no signs of brain/nerve damage/dysfunction were detected. No determination of albumin, IgG, and IgM in CSF was performed in these cases.

AUTISM GROUP (NO. 3). The third contrast group included 8 children, 5 boys and 3 girls, with clear autistic symptoms but with no other syndrome diagnosed. Their intellectual level corresponded to mild mental retardation. The mean age was 7.3 years (range 3–13 years).

MIXED NEUROLOGICAL GROUP (NO. 4). The fourth contrast group consisted of 7 children, 6 boys and 1 girl, with different diagnoses (residual state after cerebral asphyxia, herpes infection, and varicelle encephalitis, Silver–Russel syndrome, tics, and other neurologic symptoms giving rise to investigation). The mean age was 4.2 years (range 0.3–15 years). In these patients we cannot eliminate the possibility of influence on the monoamines; however, this heterogeneous group was included to obtain CSF from infants under the age of 3 years, because there were no children young enough in the other contrast groups who could be compared to the youngest children in the PWS group.

OVERWEIGHT GROUP (NO. 5). The fifth and last contrast group comprised 4 cases, all girls. They displayed the same degree of obesity and mental retardation as the PWS patients and showed some other PWS-like symptoms. Their results on the consensus diagnostic criteria were close to or at the borderline for a clinical diagnosis, but they did not have the chromosomal deletion of PWS. The mean age was 7.9 years (range 4–15 years).

The children belonging to the PWS group and contrast groups (3), (4), and (5), and the younger subjects of contrast group (1) were examined by the first author. None of the individuals in these groups had displayed epileptic seizures. All of them had an intact blood brain–barrier as judged from the spinal fluid/serum albumin ratios and no sign of any intrathecal immunoglobulin production as judged by normal spinal fluid IgG and IgM. Nobody was medicated with hormones or drugs known to affect the examined neurotransmitters.

Sampling

The CSF samples were obtained by lumbar puncture, with the syringe inserted in the L3/L4 or L4/L5 interspace after administering anesthetic salve and cleaning the skin with chloromycetin in ethanol. There was no attempt to control season of the lumbar puncture. The punctures of the PWS cases were spread out over all seasons. All cases in contrast groups (1), (3), (4), and (5) were punctured from August until May. We do not know when the

Table 1. CSF Monoamine Metabolite Levels in Various Study Groups

Variable	Group mean (SD)				p, Mantel's test
	PWS	Comparison group	n		
5-HIAA concentration	227 (53)	Healthy 1	15	95 (36)	<.001
5-HIAA concentration		Healthy 2	22	101 (33)	<.001
5-HIAA concentration		Healthy 1 + 2	37	99 (34)	<.001
5-HIAA concentration		Autism	8	128 (43)	<.001
5-HIAA concentration		Mixed neurological	7	161 (54)	.002
5-HIAA concentration		Overweight	4	156 (33)	.012
5-HIAA concentration		Autism + overweight	12	137 (41)	<.001
5-HIAA concentration		All groups	56	115 (44)	<.001
HVA concentration	531 (140)	Healthy 1	15	289 (136)	<.001
HVA concentration		Healthy 2	22	288 (86)	<.001
HVA concentration		Healthy 1 + 2	37	289 (108)	<.001
HVA concentration		Autism	8	422 (100)	.029
HVA concentration		Mixed neurological	7	406 (184)	.045
HVA concentration		Overweight	4	409 (94)	.069
HVA concentration		All groups	56	331 (129)	<.001
HMPG concentration	59 (16)	Healthy 1	15	45 (8)	.006
HMPG concentration		All groups	54	55 (13)	.282

All CSF monoamine metabolite concentrations are expressed as nmol/L.

individuals in group (2) were punctured. The punctures of the PWS cases, and of the cases in the contrast groups (1), (3), (4), and (5) were performed between 9 AM and 2 PM. About the remaining cases we have no information of the time of puncture. Some children were punctured under the effect of anesthetics. In some cases benzodiazepins were given, orally or as a rectiol. The lumbar puncture was performed within half an hour after the anesthetics or benzodiazepines were administered. If puncture bleeding appeared, the first few drops of CSF were disposed of. Samples of 3 mL were collected from children aged 0–15 years and 12 mL from older individuals. CSF was collected in a plastic tube, cooled in ice water, and immediately taken to the laboratory. The samples were centrifuged at 4°C for 10 min at 2000 × *g*, after which the supernatant was removed and frozen at −80° within 30 min after the puncture. Samples collected in other hospitals were transported on dry ice to our laboratory; however, most samples from contrast group (1), and all from contrast group (2) had been collected at room temperature and were not cooled in ice water during the collection. The cooling procedure was not required for the present study but performed to allow other analyses.

Determination of Monoamine Metabolites

All analyses were performed in our own laboratory, and all samples except those belonging to contrast group (2) were assayed by high-performance liquid chromatography (HPLC) with electrochemical detection (Blennow et al 1993a). The samples belonging to contrast group (2) were assayed with mass fragmentographic determination (gas chromatography–mass spectrometry) in a microprocedure modified from the method described by Swahn et al (1976). We have previously shown (Blennow et al 1993a) that there was no statistical difference between the two methods.

Statistics

Mantel's test (Mantel 1963) was used in the comparison of 5-HIAA, HVA, and HMPG concentrations in PWS and contrast groups (1) and (2), and PWS and the collapsed contrast groups, to partial out the effect of age. The same test was also applied for test of partial correlations between monoamine metabolites when the influence of age was eliminated. Test of correlation (nonpartial) was performed by Pitman's test (Bradley 1968), which is a nonparametric test not based on ranks. To further elucidate the correlations the Pearson's *r* was calculated as well as partial correlation coefficients. The regression between age and each one of the monoamine metabolites was estimated by a second order function. Two-tailed tests were used.

Ethics

The study was approved by the Medical Ethics Committee of the University of Göteborg.

Results

5-HIAA and HVA in PWS and Contrast Groups

Compared with all individuals in the healthy groups (1) and (2) and with the collapsed contrast groups, the concentrations of both 5-HIAA and HVA were increased in the PWS group ($p < .001$) (see Table 1, Figures 1 and 2). The differences were most clearly evident in the levels of 5-HIAA. There was no difference of 5-HIAA or HVA concentration across the different contrast groups. The HVA by 5-HIAA correlation is .65 ($p = 0.0460$) in the PWS group (partial correlation coefficient = .53, $p = .1543$) and .79, $p < .001$ (Pitman's test) in all groups

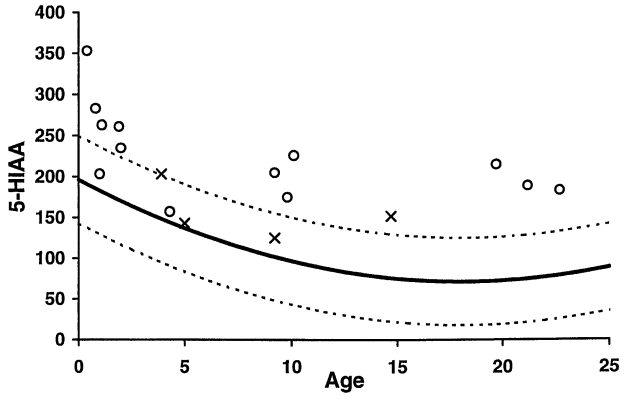

Figure 1. Concentration of 5-HIAA (nmol/L CSF) and age (years) in PWS (o) and overweight (x) individuals, compared with all other contrast groups collapsed, expressed as quadrated function of regression (bold line) with 1.96 × SD (dotted lines). 5-HIAA = 196.09 − 13.72 × age + 0.377 × age^2. Values of the PWS cases: 353, 283, 203, 263, 261, 235, 157, 205, 175, 226, 215, 189, 183. Values of the overweight cases: 203, 143, 125, 152.

collapsed (partial correlation coefficient = .70, $p < .001$). All partial correlations presented were partialled for age.

5-HIAA, HVA, and Age

For both metabolites the correlation coefficient between concentration and age was negative in the PWS as well as in all the contrast groups [r (age and 5-HIAA) = −.54, $p = .0475$ in PWS group, and = −.69, $p < .001$ in contrast groups (1), (2), (3), and (4) collapsed; r (age and HVA) = −.49, $p = .0674$ in PWS group, and = −.65, $p < .001$ in the contrast groups].

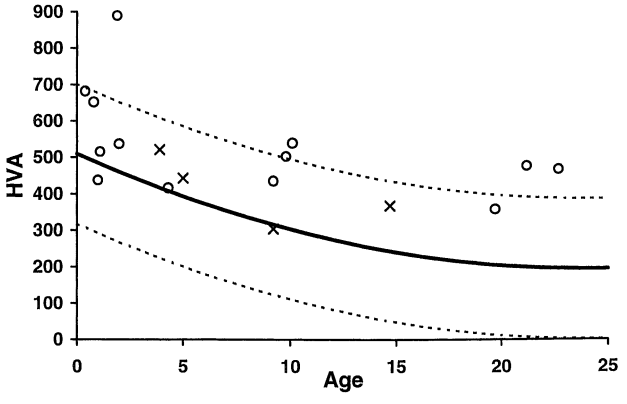

Figure 2. Concentration of HVA (nmol/L CSF) and age (years) in PWS (o) and overweight (x) individuals, compared with all other contrast groups collapsed, expressed as quadrated function of regression (bold line) with 1.96 × SD (dotted lines). HVA = 510.0 − 26.0 × age + 0.536 × age^2. Values of the PWS cases: 682, 652, 437, 515, 889, 537, 416, 435, 503, 540, 358, 477, 467. Values of the overweight cases: 521, 443, 304, 368.

HMPG and Age

The estimated correlation coefficient between HMPG and age in the PWS group was .37, $p > .30$, and in all the contrast groups −.37, $p = .0046$. The HMPG by age regression in all contrast groups except overweight was: HMPG = 60.1 + 0.079 × age − 0.040 × age^2.

5-HIAA and Body Mass Index

No clear correlation between the 5-HIAA concentration and body mass index (BMI) was seen in collapsed PWS and overweight cases aged 4 years and older ($r = −.46$, $p = .0622$, partial correlation coefficient = .15, $p > .30$).

5-HIAA and Body Height

The correlation coefficient between 5-HIAA and height was −.64, $p = .0106$, in all collapsed PWS and overweight cases. The partial correlation coefficient was −.55, $p = .0253$.

5-HIAA and Birth Weight

Within the collapsed PWS and overweight groups there was no significant correlation between birth weight and 5-HIAA ($r = −0.22$, $p > .30$). The mean birth weight in the PWS group was 2875 g and in the overweight group 3980 g. Regression analysis indicated that if birth weight had been increased even with a full 600 g, the corresponding lowering of 5-HIAA concentration would be minimally affected (5-HIAA = 256 − 0.0147 × birth weight).

5-HIAA and IQ Level

There was a significant difference in 5-HIAA concentration between the PWS group (mean 227, SD 53 nmol/L), and the two contrast groups with the same IQ level and ages (overweight and autism collapsed) (mean 137, SD 41 nmol/L, $p < .001$). The IQ variation within the PWS group was too small, and the cases too few to allow meaningful analysis of the correlation between IQ level and concentration of 5-HIAA.

HMPG in PWS and Contrast Groups

The mean concentration of HMPG was higher in the PWS group (59, SD 16 nmol/L) than in the healthy group (1) (45, SD 7.9 nmol/L, $p = .006$), but no significance was found when the PWS group was compared with the collapsed contrast groups ($p = .282$).

Discussion

All the PWS cases fulfilled the consensus diagnostic criteria (Holm et al 1993) and had the characteristic

paternal deletion or maternal disomy on chromosome 15q. Thus, findings should be generalizable to individuals with this syndrome. Recently, different phenotypic behaviors have been reported from PWS individuals with deletion and uniparental disomy (Gillessen-Kaesbach et al 1995; Cassidy et al 1997); however, since we did not perform genetic analyses that differentiate between these defects, we were not able to analyze if there are differences in monoamine levels corresponding to these variables.

According to the results of this study, PWS patients have increased CSF concentration of 5-HIAA, and to a lesser degree, of HVA. This could indicate dysfunction particularly in the metabolism of the serotonergic system in PWS. The results are in need of corroboration by other groups.

Serotonin dysfunction in PWS could be directly caused by the genetic disturbance, or the result of some kind of confounding factor.

Possible Confounding Factors

The power will be very low when studying partial correlation in small groups. In this investigation partial correlation analyses are restricted to testing with age as a background variable.

The concentration of 5-HIAA may also correlate with body build. Short, stout people are reported to have a little higher values than tall, fragile individuals (Ågren 1981). Our PWS cases were all stout and had high BMIs, and there was a correlation between 5-HIAA and body height; however, no clear correlation between 5-HIAA and BMI was seen. In a recent study (Strömbom et al 1996) on CSF monoamines in obese women, there was a negative correlation with the HVA and 5-HIAA concentrations and body weight. Low 5-HIAA concentrations were suggested to be characteristic of human obesity. The low weight at birth in our PWS group did not correlate with the elevated concentration of 5-HIAA later on. Blennow et al (1993b) studied the monoamine metabolites in a large sample of healthy individuals ($n = 114$) with a large age span (18–88 years) and could exclude seasonal and sex variances, but a negative correlation occurred with length.

In a study by Gillberg and Svennerholm (1987), high concentrations of 5-HIAA in CSF correlated with mental retardation. In the present study, however, there was a significant difference in 5-HIAA concentration between the PWS group and the other groups with the same IQ level. In fact, the concentration of 5-HIAA reported by Gillberg and Svennerholm (1987) was similar to the concentration found by us in the groups with mental retardation and similar IQ level. Thus the low IQ level alone could not explain the elevated 5-HIAA concentration in the PWS patients.

The increased levels of HVA in PWS indicate that the dopamine system may also be affected. The high correlation between 5-HIAA and HVA does not exclude a primary dopamine/HVA involvement. Dopamine is known to be important in satiety and feeding behavior (Aou et al 1994; Thombre et al 1994; Takahashi et al 1995). Dopamine is also supposed to interact with serotonin (5-HT) receptors, at least 5-HT_{2C} and possibly 5-HT_{2A} (Young et al 1993; Phillips et al 1995).

There are some studies on food preference in PWS when the subjects were allowed to select their food (Nelson et al 1981; Taylor and Caldwell 1985). A slightly higher carbohydrate intake is reported; however, our PWS cases were not allowed to have excessive amounts of their favorite foods. Therefore we do not think that the monoamine levels might be influenced by extreme differences in dietary intake.

Neurophysiological Aspects

Various serotonergic drugs are known to influence food intake, and such behavior as is frequently affected in PWS (Grignaschi and Samanin 1993; Paez and Leibowitz 1993; Hoyer et al 1994). Stimulation of the serotonin receptor 5-HT_{1A} is believed to mediate hypothermia, locomotor activity, aggression, and inhibition of nociceptive responses (Ensler et al 1993; Young et al 1993; Sánchez et al 1993; Cervo et al 1994), which are often affected in PWS.

Fenfluramine is presumed to act directly on the satiety center, thus reducing appetite (Ellinwood and Rockwell 1988). It stimulates serotonin release from hypothalamic and diencephalic areas of the brain, but also reduces the reuptake to some extent (Pinder et al 1975). Fenfluramine has been found to be useful not only for obsessive–compulsive symptoms in developmental disorders but also for hyperphagia and temper outbursts in PWS (Selikowitz et al 1990). The effects of d-fenfluramine on appetite and temperature in humans are suggested to be mediated by elevated serotonin stimulation of 5-HT_{2C} and maybe other 5-HT_2 receptors (Goodall et al 1993).

PWS patients treated with serotonin reuptake inhibitors or releasers show obvious improvement in behavior with less irritability, rigidity, temper tantrums, and compulsions such as skin picking (Dech and Budow 1991; Hellings and Warnock 1994; Greenswag 1995; Trygstad 1995).

The concentration of 5-HIAA is dependent of the metabolic rate as well as the concentration of 5-HT. Experiences from treating PWS patients with serotonin reuptake inhibitors, resulting in behavior improvement, indicate that the 5-HT transmission in PWS is low. Our high 5-HIAA levels in PWS then make us suggest that the metabolic rate of 5-HT is altered, or that the concentration

of 5-HT is high in spite of low transmission. The effect of treatment with serotonergic drugs on both obesity and characteristic behavior makes us suggest that the disturbance in serotonin turnover may be more basic or primary in the pathological mechanism in PWS.

Conclusion

The increased concentration of the serotonin metabolite 5-HIAA in CSF supports the hypothesis of an increased serotonin turnover in PWS and a decreased synaptic serotonin transmission. Many of the symptoms and behavior characteristics seen in PWS seem to be connected with the serotonergic activity. We do not know whether the increased concentration of HVA in PWS is secondary to the concentration of serotonin—and reflects the very complex regulation of satiety—or if the metabolism of dopamine has a more primary function. Maybe the higher concentrations of both 5-HIAA and HVA indicate an altered activity of the enzyme monoamine oxidase, a resistance in the receptors, or a more complex derangement responsible for the mechanisms in PWS. If the suggested serotonergic disturbance is part of the pathogenic mechanism in this complex syndrome, strictly controlled studies with pharmacologic intervention in the serotonergic system may result in new methods of treatment.

The study was supported by grants from the Swedish MRC (No. B96-21X-11251-02A for C. Gillberg, No. B96-03X-09909-05C for J.-E. Månsson, and No. B96-21X-07517-11A for R. Ekman) and from FoU Bohuslandstinget, Göteborg, the Wilhelm and Martina Lundgren Scientific Foundation, Göteborg, the Sunnerdahl Foundation, Älvsjö, and the First-of-Mayflower Committee, Stockholm, Sweden, for A. Åkefeldt.

We are grateful to the children and their parents who have helped us in this work with patience. We also thank Jan Arvidsson, Jönköping, Jürgen Bensch, Örebro, Anders Kristoffersson, Borås, Bengt Lindblad, Mölndal Mårten Kyllerman, Göteborg, and the staff at the pediatric department Kärnsjukhuset, Skövde for invaluable assistance with the sampling. We are also grateful to Karin Jansson, Annika Lekman, and Marie Molander, Mölndal for excellent technical assistance. Many thanks also to Pam Fredman and Stephan Hjort for valuable discussions, and to Anders Odén for help with the statistical analysis.

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