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Original Article

Porphyria in childhood autistic disorder is not associated with urinary creatinine deficiency

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Abstract

Background: Urinary metabolite measurements are often normalized to levels of the ubiquitous metabolite creatinine (CRT) to take account of variations in fluid export. Following CRT normalization, excesses of porphyrins and isoprostanes have been reported in the urines of children with neurodevelopmental disorders. It was suggested (Whiteley *et al.*, 2006, *Pediatr. Int.* 2006; **48**: 292–297) that urinary CRT levels may be depressed in children with autism spectrum disorders. This prompted re-evaluation of CRT levels in such children.

Methods: First matinal urinary CRT levels were compared between subjects in different diagnostic categories including autistic disorder, pervasive developmental disorder not otherwise specified (PDD-NOS) and hyperactivity, before and after correction for age and gender. A larger reference group, consisting of subjects with unrelated disorders and Asperger disorder, with no reported porphyrin excess, was also compared to the group with autistic disorder, both for CRT and for porphyrin (coproporphyrin, COPRO) excess.

Results: No significant difference in CRT was observed between any of the categories analyzed, also when corrected for age and gender. In contrast, urinary COPRO levels were significantly higher in autistic disorder versus reference groups, either when expressed as absolute values (independent of CRT levels) or when normalized to CRT.

Conclusions: These data do not support a systematic reduction in urinary CRT levels in subjects with autism spectrum disorders including autistic disorder and PDD-NOS. Urinary COPRO excess in autistic disorder was not associated with or consequent upon urinary CRT deficiency. Differences between affected and control subjects in age and sampling time, as reported by Whiteley *et al.*, may underlie the apparent CRT reduction.

Key words autism, creatinine, pervasive developmental disorder, porphyrin, urine.

Pervasive developmental disorders (PDD) of childhood include autistic disorder, PDD not otherwise specified (PDD-NOS) and the rarer conditions Asperger’s disorder and Rett disorder.^{1,2} The conditions, dubbed ‘autism spectrum disorders’ (ASD),^{3,4} are characterized by deficits in social interaction, impairments of speech and language, and restricted behavioral repertoire. Related conditions include attention-deficit disorder, sometimes with hyperactivity, common comorbidities of ASD.

Behavioral features are often accompanied by subtle physiological dysregulation, including immune system and gastrointestinal anomalies. At the biochemical level, irregularities have been seen in blood serotonin,^{5,6} steroid and polypeptide hormones including cortisol, dehydroepiandrosterone, oxytocin, and testosterone,^{7–11} and in sulfur metabolite levels^{11–13} as reviewed.¹⁴

For communication-impaired subjects, non-invasive methods of evaluation are important, including analysis of hair and urine. For the latter, careful normalization is required because volume output (and metabolite concentration) fluctuates according to

diet, water intake, and time of day. Urinary metabolite levels are commonly normalized to urine levels of a nitrogenous waste product, creatinine (CRT), although the need for CRT normalization has been debated.^{15–17} CRT is predominantly (95%) derived from muscle; urinary levels of CRT reflect lean body mass^{18,19} and excretion rates correlate strictly with musculature²⁰ and therefore with age and height.²¹ The correlation coefficient with lean body mass was 0.988.¹⁹

Following CRT normalization, excesses of porphyrin and isoprostanes have been observed in the urines of children with autistic disorder.^{22,23} Nevertheless, the reliability of these data could be undermined if CRT levels were significantly different in autistic children versus controls. Although no systematic changes in body mass/musculature have been described in ASD, Whiteley *et al.* recently reported a mean 35% reduction in urinary CRT in children with ASD (PDD);²⁴ if confirmed, this could complicate measures based on CRT normalization of urinary metabolites in children with neurodevelopmental disorders.

To address the possibility that urinary CRT decline might underlie the porphyrin excess previously reported,²² post-hoc analysis of urinary CRT values was performed in a large group of French children with autism and related neurodevelopmental disorders.

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Methods

Study groups

This study used a subset ($n = 217$) of children presenting to the Clinique Dr Skorupka, Paris, between August 2002 and December 2004 described previously.²² The target study group was all children with a strict diagnosis of autistic disorder for whom primary CRT values were documented ($n = 100$, mean age 6.5 years, age range 2–15 years, 23% female). Other categories studied included Asperger's disorder ($n = 11$, mean age 10 years, range 5–15 years, 9.1% female), attention deficit disorder ($n = 7$, mean age 9 years, range 6–14 years, 85.7% female); autism with known epilepsy ($n = 8$, mean age 9 years, range 5–14 years, 25% female); cerebral palsy ($n = 11$, mean age 8.4 years, range 6–15 years, 45.5% female); hyperactivity ($n = 25$, mean age 9.7 years, range 3–14 years, 4% female); PDD-NOS ($n = 43$, mean age 6.6 years, range 2–15 years, 20.9% female). The control group consisted of children with unrelated disorders ($n = 12$, mean age 10.3 years, range 4–16 years, 41.7% female). Diagnostic criteria were as reported previously.²² Because of the limited number of children in the control group, to improve statistical power, plots of CRT against age (and absolute porphyrin levels) were also compared for all children with autistic disorder, for whom porphyrin excess has been described, against a larger reference group consisting of control children plus children with Asperger's disorder (in total, $n = 23$, mean age 10.1 years, range 2–15 years, 26.1% female), for whom previously no porphyrin excess was observed.²²

Urinary creatinine measurement

Methods were as previously described.²² First matinal urines (10 mL) were stored in the dark (<2 days, ambient temperature) and then frozen (-20°C). Urinary CRT levels were determined using a spectrophotometric enzyme-linked assay described by the instrument supplier (Crea-Vitros Technical Bulletin; Ortho-Clinical Diagnostics, Johnson and Johnson, High Wycombe, UK); CRT reference standard was from the same source.

Age normalization

To take account of age variation in CRT excretion, where levels approximately double over the 2–15 age range, rising linearly at best approximation from 640 mg/L (age 2–3 years) to 1316 at age 14–15 years (5.66–11.65 mmol/L),²² data for the autistic group and the reference group (control plus Asperger's disorder) were plotted against age, a linear regression curve calculated (Excel, Microsoft, Redmond) and age 10 intercepts used as before²² to compare the age-normalized CRT levels between groups.

Statistical analysis

Analyses (mean, SD, linear regression) used Excel (Microsoft Corp.) and Student's *t*-test, two-tailed, unequal standard deviations, Satterthwaite approximation (GenStat; VSN International, Hemel Hempstead, Herts, UK). Post-hoc anonymous analysis of urinary metabolites was approved by the NHS Lothian Local Research Committee O4 (Scotland).

Results

To address whether porphyrin excess reported in children with autistic disorder might be linked to urinary CRT deficiency, first matinal urinary CRT concentrations were recorded in a large group of French children with autism and related neurodevelopmental disorders. Levels in groups with no evident porphyrin excess (control children and Asperger's disorder) were compared to those seen in autistic disorder, where porphyrin excess was observed. Other conditions studied included attention deficit, cerebral palsy, hyperactivity, and PDD-NOS; the separate category of autistic disorder plus epilepsy was included because anti-epileptic medication is a potential complicating factor. CRT levels are presented in Figure 1.

Despite variation in mean values, no statistically significant differences were observed between any of the groups, although there was a trend to reduced CRT in autistic children as well as in individuals with attention deficit and cerebral palsy. The statistical significance of the difference between the means of the highest and lowest groups (autism with epilepsy, vs cerebral palsy) was only $P = 0.26$, but the groups differed slightly in age.

CRT levels alter with age, rising approximately linearly from age 2 to age 16. To evaluate whether age differences might account for the difference, levels were re-plotted against age. On first analysis there was no significant or discernable difference between any of the groups. To increase overall statistical significance, autistic disorder (with porphyrin excess) was compared against a larger reference group consisting of control and Asperger disorder children (for whom no porphyrin excess was

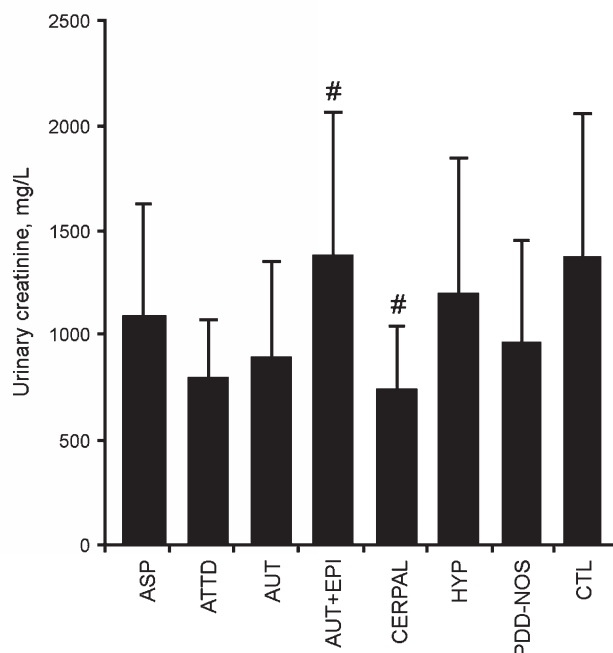


Fig. 1 Urinary creatinine levels (\pm SD). ASP, Asperger's disorder; ATTD, attention deficit; AUT, autistic disorder; AUT+EPI, autistic disorder with known epilepsy; CERPAL, cerebral palsy; CTL, control; HYP, hyperactivity; PDD-NOS, pervasive developmental disorder, not otherwise specified. The statistical significance of the difference between the highest and lowest groups (#) was $P = 0.26$.

recorded in a previous study) using a scatter plot; best fit regression lines were drawn, and age-10 intercepts calculated.

Intercepts were approximately 1300 mg/L in control children and approximately 1100 mg/L in children with autistic disorder (Fig. 2). Despite this small depression in the age 10 intercepts in ASD versus the reference group (approx. 15%), neither the values nor the gradients of the regression lines were significantly different. The calculated significance level was $P = 0.57$.

The CRT levels for younger children (<7 years) in the autistic disorder group appeared somewhat higher than those for the reference group (where reference values all lie below the fitted line for their group), but this also did not achieve statistical significance. There were small differences in the distribution of genders between groups. Across the entire study group CRT levels were depressed by approximately 18% in female versus male subjects (data not shown), presumed to reflect gender dimorphism of stature and body mass. When figures were specifically adjusted to take this into account no significant difference was observed between the adjusted and unadjusted mean levels (not shown). These data thus provide no evidence for systematic differences in urinary CRT levels between these study groups.

To confirm formally that previously reported porphyrin excess in autistic disorder²² is not linked to differences in CRT levels, absolute coproporphyrin (COPRO) levels were compared between autistic disorder and the larger reference group (Asperger and control). The excess was reiterated in absolute COPRO levels, despite greater scatter (apparent shift of medians towards baseline) due to individual differences in fluid output, and was

statistically significant (autistic disorder vs reference group; $P = 0.02$; Fig. 3a). The difference in CRT-normalized COPRO values between these groups was also significant ($P = 0.01$; Fig. 3b), in accordance with previous results.²²

Discussion

It was suggested that CRT levels could be reduced in children with PDD including autistic disorder.²⁴ The present data do not support this reduction. No statistically significant difference in CRT levels was observed between any of the groups studied here. When CRT levels in autistic disorder, with porphyrin excess,²² were compared with those of a reference group with no porphyrin excess, and allowing for age-differences in the study groups, there was no significant difference between the normalized CRT values for the two groups. Further, the porphyrin excess reported in autistic disorder²² was maintained in comparison of absolute values without reference to CRT levels.

Creatinine is primarily derived from muscle. Impaired growth or reduced muscle mass is generally not seen in children with ASD, although caution is needed because growth impairment is one outcome of early life central nervous system injury.²⁵ In an early study in Japan on 42 subjects with autistic disorder, overall height did not differ from controls, although autistic boys aged 6–12 years appeared taller.²⁶ Reduced body mass index (BMI) has been described in children with autism²⁷ but the link was inconsistent and could be linked to hyperactivity,²⁸ sometimes comorbid with ASD. More generally, studies have pointed to raised BMI in autism and related neurodevelopmental disorders;²⁹ obesity was recorded in a proportion. In 42 US children with ASD (predominantly autistic disorder and PDD-NOS) there was a non-significant trend towards increased BMI versus

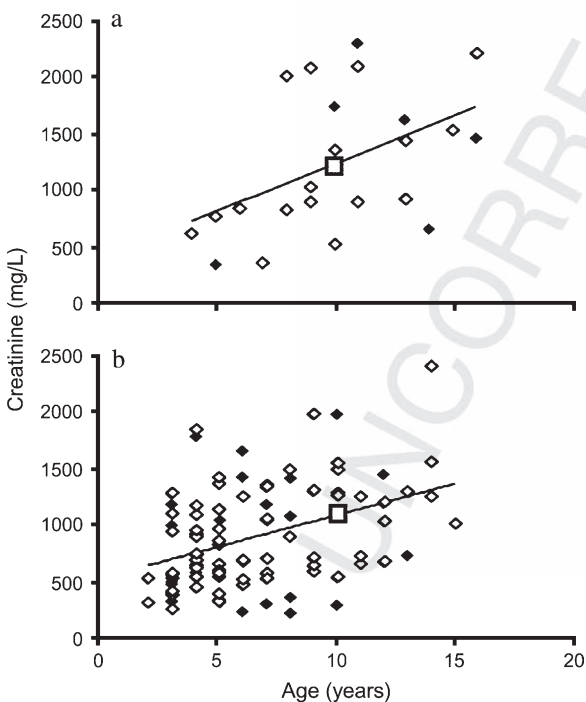


Fig. 2 Urinary creatinine levels in (a) reference and (b) autistic disorder children plotted as a function of age. (\diamond) boys; (\blacklozenge) girls. The reference group consisted of control children (unrelated disorders) and Asperger's disorder for whom no porphyrin excess was reported. Inter-group statistical significance was $P = 0.57$.

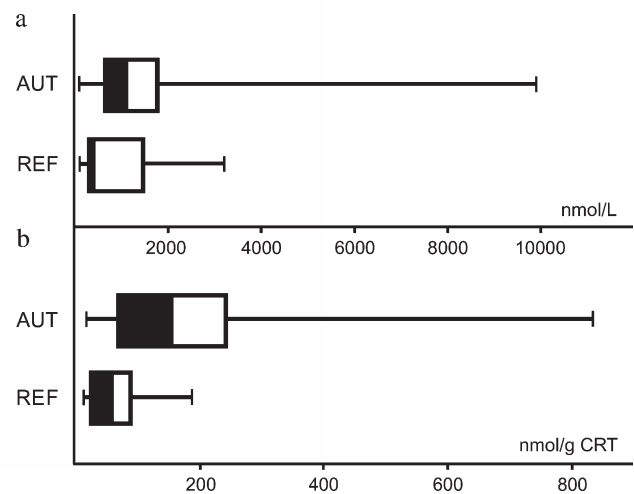


Fig. 3 Coproporphyrin levels in autistic disorder (AUT) and reference group subjects (REF). (a) Absolute urinary levels in nmol/L; (b) values normalized to urinary creatinine (CRT) in nmol/g CRT. Verticals are minima, maxima, and quartile medians. The midway medians in both cases are threefold higher than in the REF subjects despite greater scatter in the un-normalized values. Inter-group statistical significances were $P = 0.02$ (a; absolute coproporphyrin) and $P = 0.01$ (b; CRT-normalized coproporphyrin levels).

population reference ranges.³⁰ In the primary study suggesting CRT deficiency the study subjects were on average in the upper 50%,²⁴ confirming an absence of obvious growth deficiency. Reduced muscle mass (with concomitant reduction in urinary CRT levels) may be unlikely in the absence of growth retardation.

Only one independent study has specifically examined CRT levels in such children, and in that study no difference was found between children with autistic disorder and healthy controls of the same age,²³ indeed that study reported a slight but not significant CRT excess in children with autistic disorder. Other reports support the absence of a CRT bias. In a study on urinary indolyl-3-acryloyl-glycine in ASD, no excess was found either in unnormalized concentrations or when concentrations were normalized to CRT:³¹ the absence of excess on CRT normalization would seem to preclude systematic deficiency of urinary CRT in the children studied. Excess of urinary porphyrins in autistic disorder was also reported either in CRT-normalized data or in internal ratios independent of CRT values,²² pointing away from systematic depression of CRT levels in autism. A further study has pointed to porphyrin excess without CRT normalization.³²

Deficiency in the X-linked creatine transporter gene, although uncommon, has been linked to an autism-like behavioral disorder in some subjects. Although brain creatine levels are depleted, blood creatine levels are elevated³³ and, because creatine is converted to CRT for renal disposal, elevation (rather than depression) of urinary CRT would appear likely in these subjects. However, the relative rarity of such disorders (up to 1% of male subjects with mental retardation of unknown etiology³⁴) argues that brain creatine transporter deficiency is unrepresentative of the large majority of ASD cases.

Other factors may explain the apparent reduction of urinary CRT reported²⁴ in subjects with PDD, including autistic disorder. While dietary differences between the PDD and control groups are not excluded, two specific factors emerge. First, urinary CRT levels increase with age; the median age of the PDD group was younger (75 months) than the median age of the control group (110 months).²⁴ Adjusting for age, a mean CRT decline of approximately 15% is expected in the PDD group on this factor alone.

Second, as properly highlighted by Whiteley *et al.*, there was a difference in sampling time between the control (first matinal urines) and the PDD (12.00–16.00h) groups that could explain some of the discrepancy.²⁴ Few systematic studies on diurnal CRT variation have been reported, but a decline in afternoon versus morning urinary CRT has been described, ranging from 4 to 20%,^{35,36} although those studies were in adults and the latter study addressed rates rather than concentrations. Martin *et al.*, also working with adults, reported CRT elevation in early morning urines with midday depression; the difference amounted to 50% variation around the baseline.³⁷ Although that diurnal change was not fully reproduced in a subsequent study,³⁸ it is plausible to suggest that the depression in the childhood PDD group, sampled later in the day than controls in the Whiteley *et al.*²⁴ study, is a reflection of different specimen collection times. In the present report, as before,²² urines were collected at constant time of day.

Whiteley *et al.* also reported a significant difference in the mean pH of urines from PDD versus control children.²⁴ Urinary

pH varies according to time of day, and although the explanation is open to debate, a so-called alkaline tide (a post-prandial rise of urinary pH) is well documented.^{39–41} The reported elevation of urinary pH in the PDD group (12.00–16.00h sampling) versus controls (first matinal) would appear to be accounted for by this effect, while also corroborating the difference in sampling times.

The urinary porphyrin excess previously recorded in children with autistic disorder may be suggestive of exposure to environmental toxins, including heavy metals and or chemical toxicants, that inhibit different steps in the heme synthesis pathway, so leading to excretion of porphyrin intermediates.^{22,42,43} Elevated body heavy metal burden,⁴⁴ with reported excesses in teeth,⁴⁵ is consistent with impaired capacity to export toxic metals.^{46,47} From this perspective, CRT normalization could underestimate the true extent of urinary porphyrin excess in these subjects because urinary CRT levels tend to rise in adult subjects exposed to mercury,⁴⁸ although children have not been studied. Nevertheless, we detect no systematic differences in urinary CRT levels between children with autistic disorder and controls, although the present data do not rule out a weak trend towards reduced urinary CRT (or increased, particularly in the younger subjects) in children with ASD. In conclusion, the present data do not substantiate systematic depression of urinary CRT in autistic disorder: previously reported excesses of urinary porphyrins²² and isoprostanates²³ after CRT normalization would not appear to be consequent upon or associated with bias in CRT levels.

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