Observed activities of serum creatine kinase: total and B subunit activity and other enzymes in young persons abusing solvents

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SUMMARY Serum enzymes (aspartate transaminase, alanine transaminase, alkaline phosphatase (ALP), γ -glutamyltransferase, and creatine kinase (CK)) were measured in 296 young persons who admitted to recent inhalation of solvents, usually toluene based glues. In general, results fell within expected adult reference ranges except for ALP and CK. About 60% of subjects had CK activities above the upper reference limit and these activities were investigated in terms of their isoenzyme composition.

CK B subunit activity was measured in 90 subjects with raised total CK activities. In five instances the CK B subunit activity was judged abnormal and in two subjects the presence of CK BB was confirmed. These two subjects were thought to have a circulating macro CK, type 1.

It is concluded that the increased total CK activity found in this group of solvent abusers was due to physical activity, but a contribution from specific muscle toxicity by solvents cannot be excluded.

Solvent abuse among young persons has been widely described in the media and popular press and in professional journals. As with any substance abuse there can be social, psychological, and physical complications. The full extent of the last of these is not yet known, although specific problems such as encephalopathy,¹ epileptiform attack,² and hepato-renal damage³ have been reported in the UK and a range of uncommon serious toxic effects have been reported in North America.⁴

The prevalence of solvent abuse is such that examination of abusers must continue so that more knowledge is gathered about morbidity. Biochemical organ profiling plays an important role in this respect.

Information on reference ranges for serum enzymes in this young age group is limited. Moreover, it may be outdated following the recent introduction of new methods for their measurement

recommended by national societies. General trends may be drawn, however, from the published work.

Creatine kinase (CK) is particularly sensitive to exercise,⁵ and previous workers reporting CK activities in normal children have assumed that raised values are due to recent physical activity,^{6,7} repeat samples showing a reduction in enzyme activity.⁷ The observed increases in CK activity in this age group have never been explained in terms of the isoenzymes of CK.

In 1979 a psychiatric outpatient department was introduced specifically for the referral of young persons abusing solvents. We took the opportunity to study the activities of a number of serum enzymes in this population.

Subjects and methods

Between November 1979 and March 1983 296 young persons (263 male and 33 female) aged between 7 years 11 months and 19 years 4 months were referred by the authorities, usually the police, or parents to a psychiatric outpatient clinic at the London Road Police Office, Glasgow, for counselling following solvent abuse. The subjects, accompanied

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Accepted for publication 15 December 1983

by their parents or guardians, were interviewed and counselled by a community police officer and then by a psychiatrist, who, with informed consent, took a blood sample for the purpose of detecting sub-clinical disease. All subjects were of average health.

Thirty six subjects were seen on more than one occasion, usually within eight weeks of first attending. Three subjects were seen again after a year and one after three years. During the study two subjects who were severely intoxicated and suffering from self neglect and lack of supervision were admitted to a psychiatric hospital and a few others received outpatient treatment related to family problems.

Samples were analysed for aspartate transaminase (AST; EC 2.6.1.1),⁸ alanine transaminase (ALT; EC 2.6.1.2),⁸ alkaline phosphatase (ALP; EC 3.1.3.1),⁹ γ -glutamyltransferase (GGT; EC 2.3.2.2),¹⁰ and creatine kinase (EC 2.7.3.2).¹¹ All analyses were performed at 37°C using a Centrifichem 400 (Baker Instruments, USA).

CK B subunit activity was determined on a LKB 2086 Reaction Rate Analyser (LKB, Sweden) with blank subtraction as recommended by the Scandinavian Committee on Enzymes.¹² Reagents were supplied in kit form from E Merck (Darmstadt, FRG).

As the clinic is held in the evening, samples were stored as whole blood at ambient temperature and delivered to the laboratory the next morning. The effect such storage might have on enzyme activities was studied by taking 38 duplicate samples. One sample was centrifuged and the serum analysed promptly, while the other was stored unseparated for a further 18 h. Statistical analyses of the paired results showed AST and ALT to be reduced by 2 U/l and 3 U/l respectively ($p < 0.01$) at mean activities of 50 U/l and 30 U/l respectively after overnight storage of whole blood.

A separate experiment showed there was no loss of CK B subunit activity in 10 samples treated similarly.

Results

AST, ALT, ALP, AND GGT

Table 1 summarises the results for these enzymes.

Table 1 Serum enzyme activities in 263 males and 33 females abusing solvents

	AST		ALT		ALP		GGT	
	Male	Female	Male	Female	Male	Female	Male	Female
Median activity (U/l)	27	22	18	15	493	318	13	10
Age relation (r)	-0.185*	NS	NS	NS	NS	-0.523†	0.302†	NS
Skewed distribution	Yes	No	Yes	No	Yes	Yes	Yes	No
95% confidence interval (U/l)	9-45	9-35	4-30	8-30	145-1000	130-820	9-25	4-20

NS = no significant correlation.

* $p < 0.05$; † $p < 0.001$.

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Fig. 1 Distribution of total creatine kinase (CK) activity in the serum of 263 males and 33 females abusing solvents. CK activity of samples from normal male and female subjects are also shown.

The distributions were tested for skewness using the equations given by Snedecor.¹³ One subject had an increased AST activity and two had increased ALT activities as judged by adult reference ranges. This compares with the finding of no abnormalities of aminotransferase activity in a series of 115 subjects.¹⁴ The highest ALP activity was 1775 U/l with seven other values > 1000 U/l, results in agreement with published data.¹⁵ No abnormal GGT values were found. The association of GGT activity with age in male subjects has been reported previously,¹⁶ and concurrent alcohol abuse seems the most likely explanation in our subjects.

CK

The distribution of total CK activities found in both sexes is shown in Fig. 1. This figure also includes results for children aged between 8 years and 15 years obtained from blood samples submitted for determination of cholinesterase genotype; we were unable to obtain a suitable control population from subjects not attending hospital.

Statistical analysis of the data is shown in Table 2. Four CK activities > 600 U/l were found, the highest being 870 U/l, and all are included in the statis-

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Table 2 Creatine kinase activities in 263 males and 33 females abusing solvents

	Male	Female
Median activity (U/l)	185	142
Age relation (r)	0.111*	-0.275*
Skewed distribution	Yes	Yes
95% confidence interval (U/l)	45-420	80-240

*No significant correlation.

tics. The negative association between CK and age seen in females, although not significant, is in keeping with the findings of Smith,⁷ who showed an oestrogen dependent reduction in CK at the menarche.

CK activity greater than 200 U/l was found in 119 subjects (40%), comprising 117 males and two females. Whenever possible CK B subunit activity was measured on these samples, and a positive correlation ($p < 0.001$; Kendall Rank) between total CK and B subunit activity was found (fig. 2).

Fig. 2 contains a discriminant for abnormal CK B subunit activity. This discriminant combines the recommendations of the kit manufacturer (CK B subunit activity should be $< 3\%$ of total CK activity) and those of the Scandinavian Committee on Enzymes (CK B should be < 12 U/l at 37°C)^{12,17} and encompasses the 97% confidence limits for a normal population.

On the basis of this discriminant five subjects were identified as having raised circulating CK B subunit activities. Further samples were obtained from four of these and additional investigations are detailed in Table 3. In three subjects the presence of a raised CK B subunit activity was confirmed using an immunoinhibition technique with a kinetic bioluminescent end point (LKB, Sweden). The sample from the fourth subject was not available at this time. Using electrophoresis on cellulose acetate¹⁸ it was only possible to visualise CK MM in all four subjects.

Specific radioimmunoassays for CK MB and CK BB¹⁹ showed increased circulating CK BB in two subjects. The thermal inactivation test proposed for

Fig. 2 Association between total creatine kinase (CK) and CK B subunit activity in the serum of 90 young persons abusing solvents. Circles represent subjects thought to have increased CK B activity; the closed circle represents the subject not available for follow up. Broken line represents the discriminant; CK B = 12 U/l or 3% of the total CK activity.

the detection of macro CK complexes²⁰ was unsatisfactory owing to the low initial activities.

Discussion

It is unusual for CK activities to be requested in children, the notable exception being in the investigation of muscular dystrophy. Here detection of carrier state requires well documented reference ranges. The published work, however, contains conflicting reports concerning the age and sex relations of CK.

It is accepted that CK activities are higher in neonates than in children and adolescents.^{17,21} Ranges derived for children and young persons either show close adherence to the adult reference range²¹ or show larger variations in activity, particularly in males,⁶ similar to the findings of the present study. Where ranges are derived from subjects attending hospitals²¹ it is likely that many of the demographic variables which might affect plasma constituents of a typical solvent abuser have been

Table 3 Further investigations of subjects with abnormal creatinine kinase B subunit activities

Patient no	Date	Total CK (U/l)	CK B (U/l)	CK B (as % of total)	CK MB* (ng/ml)	CK BB† (ng/ml)
1	6.1.81	545	Insuff	—		
	9.2.81	291	13	4.5		
2	18.8.81	199	14	7		10
	4.9.81	177	15	8.5		
3	30.3.82	294	14	4.8		
	26.4.82	179	8	4.5		
4	24.6.80	220	32	14.6		
	9.7.80	143	21	14.7	5.5	4

CK = creatinine kinase.

*Normal < 50 ng/ml.

†Normal < 3 ng/ml.

Insuff = insufficient sample for analysis on this occasion.

removed. In our study all subjects had been referred for counselling following recent abuse of solvents and, although judged to be in good health by the examining physician, they cannot be regarded as strictly normal. The results of the study do give guidelines as to the expected values in this age group. In the absence of a truly representative control group, we thought it worthwhile to confirm that the increases in total CK activity were due to increased CK MM isoenzyme.

The results show that only two subjects had demonstrable increases in an isoenzyme of CK other than MM and three others were thought to have abnormal B subunit activities. Most of the subjects with apparently raised CK activities had B subunit activities < 3%, consistent with the observation that skeletal muscle contains a small percentage of CK MB isoenzyme²² and supporting the suggestion that release of CK from muscle by physical activity is responsible for the raised CK activities seen.

The most likely form of circulating B subunit in normal subjects is CK BB in the form of a macro CK, type 1.¹⁷ Macro CK, type 1, a CK BB immunoglobulin complex, has not yet been associated with any disease. It is found in up to 6% of patients in hospital,¹⁷ often without an associated increase in total CK activity. In our population macro CK occurred in at least 2% and may have been as high as 11% if all five subjects considered to have an increased B subunit activity are included. Macro CK is commonest in elderly women¹⁷ but its incidence in young persons has not been previously assessed.

There is an inverse relation between CK BB and age;^{23, 24} and with one report (using a specific radioimmunoassay) showing CK BB to be higher in 14 year old than in 8 year old and 25 year old subjects, the incidence of suspected macro CK in our population may be related to the natural distribution of CK BB.

Although the increases in CK found in young persons abusing solvents are primarily the CK MM isoenzyme, this does not eliminate the possibility that some component of the increase is due to solvent related muscle injury. The increases in CK following acute alcohol ingestion are due to CK MM.²⁵

This report confirms the findings of Watson¹⁴ that biochemical monitoring of solvent abusers does not show serious biochemical abnormalities. With the increasing evidence of specific lesions, however,¹⁻³ it is important that biochemical surveillance is maintained.

We thank Dr S Rosalki for help with the electrophoresis of samples Dr CP Price for arranging the specific radioimmunoassays, Dr WHS Thomson

Spooner, Birrell, McLelland, Baird, Sourindhrin for useful discussion, and Mr Patrick Hamill QPM, Chief Constable, Strathclyde Police Force, for his cooperation in the running and staffing of the Solvent Abuse Clinic.

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