

## Infants in Trinidad excrete more 5-L-oxoproline (L-pyroglutamic acid) in urine than infants in England: an environmental not ethnic difference

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The demand for glycine to satisfy normal growth during early life is considerable and most has to be made endogenously. The extent to which adequate glycine is available can be assessed by measuring the urinary excretion of 5-L-oxoproline. The excretion of 5-L-oxoproline at 6 weeks of age for infants in Trinidad of African, Indian or mixed parentage (398  $\mu\text{mol}/\text{mmol}$  creatinine) was significantly greater than for infants born in England of Caucasian parentage (194  $\mu\text{mol}/\text{mmol}$  creatinine). There was no relationship between 5-L-oxoproline excretion and either sex or pattern of feeding. There were significant inverse relationships between 5-L-oxoproline/creatinine and birth weight, and head circumference either at birth or 6 weeks of age, suggesting that limited availability of glycine is associated with poorer growth before and after birth. For a group of infants born in England of Indian parentage, excretion of 5-L-oxoproline (155  $\mu\text{mol}/\text{mmol}$  creatinine) was not different to infants of Caucasian parentage, but significantly less than infants born in Trinidad. The demonstration that 5-L-oxoproline/creatinine was similar in infants born in England, regardless of parentage, shows that the differences between England and Trinidad are related to environment and are unlikely to be accounted for by genetic differences or ethnicity.

### 5-L-Oxoproline: Glycine: Growth

Constrained growth during fetal and infant life is associated with increased risk of chronic non-communicable disease such as hypertension, heart disease and diabetes mellitus during adulthood (Barker *et al.* 1993; Barker, 1994). It is proposed that metabolic competence is programmed by nutrient exposure of the fetus *in utero* and this determines the ability to cope with a range of environmental factors at later ages (Wootton & Jackson, 1996). The differences are seen across cultures and may explain the emerging epidemic of diabetes and heart disease seen in people from the Indian subcontinent (Miller *et al.* 1989) and other parts of the developing world (World Health Organization, 1996). Essential hypertension is common in Jamaica (Wilks *et al.* 1996). In a prospective study of pregnant women in Jamaica, the body composition of the mother during pregnancy, weight gain during pregnancy and her haemoglobin level during pregnancy were closely associated with the blood pressure in her child at eleven years of age (Godfrey *et al.* 1994). In a retrospective cohort study of 2337 children aged 6–16 years in Jamaica, systolic blood pressure was inversely related to birth weight. Children who had been shorter and fatter at birth had higher levels of glycated haemoglobin and serum cholesterol was higher in those who were shorter at birth and the at time of study (Forrester *et al.*

1996). These studies link early growth to a metabolic profile which predisposes to chronic disease.

Growth is a complex process and the partitioning of nutrients is determined by a number of factors. However, there is an absolute requirement for protein and amino acids for net deposition of lean tissue (Jackson & Wootton, 1990). Of all the amino acids, the ability to form adequate amounts of glycine is likely to be the most critical during fetal and infant life (Jackson, 1989, 1991, 1995). As well as the formation of intracellular protein, glycine is required in large amounts for extracellular proteins such as collagen and elastin and is consumed in the synthesis of haem, creatinine, nucleotides and bile salts (Neuberger, 1981). In preterm infants, glycine is a conditionally essential amino acid and when its availability is marginal can constrain the rate of lean tissue growth (Jackson *et al.* 1981).

The urinary excretion of 5-L-oxoproline provides an indirect marker of the extent to which endogenous formation is able to satisfy the requirement for glycine (Jackson *et al.* 1987). In normal adults there is a low level of excretion of 5-L-oxoproline, an intermediary in the  $\gamma$ -glutamyl cycle of glutathione metabolism, in the urine. Urinary excretion of 5-L-oxoproline is increased when the glycine pool is depleted, through conjugation

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with sodium benzoate to form hippuric acid, or on low-protein diets when the endogenous formation of glycine in the body falls (Jackson *et al.* 1987, 1996). The excretion of 5-L-oxoproline is increased during normal pregnancy (Persaud *et al.* 1989; Jackson *et al.* 1997b). Compared with adults, excretion is increased in normal newborn infants and is especially high in preterm infants during the first weeks of life (Jackson *et al.* 1997a). The pattern of excretion in pregnancy and infancy differs between England and Jamaica. In Jamaica, excretion of 5-L-oxoproline increases progressively as pregnancy advances and by the third trimester is twice the excretion of women in England (Jackson *et al.* 1997b). The pattern is similar in infancy, with excretion being the same in neonates in England and Jamaica at 2 weeks of age, but increasing progressively in infants in Jamaica with the result that by 6 weeks of age excretion is twice that found in infants in England (Persaud *et al.* 1997). There are many factors which might account for these differences. However, as the subjects studied in Jamaica were of African origin and those studied in England were Caucasian, ethnic or genetic differences have to be considered. In Trinidad about half the population is of African origin and half of Indian origin. In the present study the excretion of 5-L-oxoproline in urine has been measured in infants from these groups. The objective was to determine whether there was any difference between infants of African or Indian descent in Trinidad, or between babies in Trinidad and in England.

### Subjects and methods

Single samples of urine were collected in a sterile urine bag from infants aged between 28 and 42 d in Trinidad and in England. The samples were acidified with 6 M-HCl and stored below  $-4^{\circ}$ . Samples taken in Trinidad were transported to England, frozen in dry ice, for analysis. The study had ethical approval from the appropriate authorities in Trinidad and Southampton, England.

Mothers who had recently delivered at the Mount Hope Women's Hospital, Trinidad, were invited to participate by

post. They were asked to provide a specimen of urine from the baby when they attended a postnatal clinic. Mothers who had recently delivered at the Princess Anne Maternity Hospital, Southampton, England were contacted by telephone and a urine sample was collected during a home visit.

Mothers were asked the age and pattern of feeding for their infant. The occipito-frontal head circumference and weight of each infant was measured using a standard method. The hospital records were used to determine details of the maternal obstetric and medical history, maternal haemoglobin during pregnancy, the duration of pregnancy and any complications, weight and head circumference of the infant at birth, and the ethnic group of the infant.

A three-step method was used to measure urinary 5-L-oxoproline (Jackson *et al.* 1996). Short ion-exchange-chromatography was used to isolate 5-oxoproline from about 2 ml urine. The oxoproline was converted to glutamic acid by hot-acid hydrolysis. L-Glutamic acid was determined enzymically using glutamate dehydrogenase (EC 1.4.1.2). Urinary creatinine was determined by the alkaline picrate method, following pretreatment of the urine with Fuller's earth to remove interfering chromogens. The urinary excretion of 5-L-oxoproline was expressed as a ratio to the excretion of creatinine.

Statistical analysis was carried out using SPSS. The Kolmogorov-Smirnov goodness of fitness test was used to assess normality of distribution and the log transformation of 5-L-oxoproline/creatinine was used. Comparisons between groups were made using unpaired Student's *t* test and ANOVA with *post hoc* determination of between-group differences, using Duncan's multiple range test. Linear regression analysis and multiple linear regression analysis were used to compare the relationships amongst different variables.

### Results

A total of forty-two infants were studied in Trinidad and twenty-two infants in England. As shown in Table 1, the Afro-Trinidadian mothers were significantly younger than either the English Caucasian or Indo-Trinidadian mothers,

**Table 1.** Maternal age, parity and haemoglobin and the weight and head circumference of their infants at birth and at about 6 weeks of age when urinary excretion of 5-L-oxoproline was measured in four groups of infants, three from Trinidad and one from England

	England		Trinidad				P value		
	Caucasian (n 22)		Indian (n 17)		African (n 15)	Mixed (n 10)			
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	
Maternal age (years)	28.1	4.2	28.2	6.3	24.3*‡	3.8	26.8	4.4	0.088
Parity	2.2	1.4	2.5	1.6	1.8	1.1	1.7	0.9	0.89
Maternal haemoglobin (g/l)	11.5	1.0	11.2	1.7	11.4	0.8	11.2	1.3	0.33
Birth head circumference (mm)	342	16	323*	20	328*	15	323*	12	0.0013
Birth weight (kg)	3.45	0.49	2.68*	0.63	3.04*	0.62	2.68*	0.6	0.0015
Head circumference (mm)	379	12	359*	20	362*	15	356*	17	0.0001
Weight (kg)	4.45	0.38	3.93*	0.79	4.45	1.05	4.18	0.5	0.103
Weight change (kg)	1.01	0.39	1.13	0.44	1.48*†	0.53	1.50*	0.5	0.0074
Head circumference change (mm)	37	12	38	15	35	20	34	17	0.87
5-L-oxoproline ( $\mu$ mol/mmol creatinine)	194	78	431*	165	324*†	111	456*‡	206	0.0000

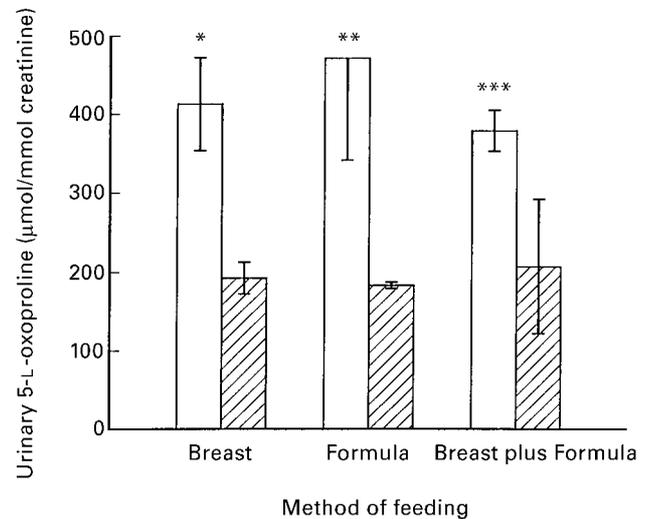
ANOVA, *post hoc* Duncan's multiple range test: significantly different  $P < 0.05$ , \* English Caucasian, † Trinidad Indian, ‡ Trinidad African.

but there were no statistically significant differences in parity or maternal haemoglobin between the groups.

For all the infants born in Trinidad, weight at birth (2.84 (SD 0.61) kg) was significantly less than for infants born in England (3.45 (SD 0.49) kg;  $P = 0.000$ ) and head circumference was also less (325 (SD 16) mm v. 342 (SD 16) mm;  $P = 0.000$ ). Amongst the groups in Trinidad there were no differences in head circumference or weight at birth, but each group had a statistically significantly smaller head circumference and was lighter than the English Caucasian group (Table 1). The difference in head circumference was still present at the time of study (Trinidad, 359 (SD 17) mm; England, 379 (SD 12) mm;  $P = 0.000$ ), but the differences in weight were no longer present except for the Indo-Trinidadian group in which weight was significantly less than the English Caucasian group at this time (Table 1). Thus the increase in weight after birth was almost 50% greater in the Afro-Trinidadian and mixed Trinidadian group than the Indo-Trinidadian and English Caucasian groups (statistically significant differences).

There was a highly statistically significant difference in the urinary excretion of 5-L-oxoproline between infants in Trinidad (398 (SD 166)  $\mu\text{mol}/\text{mmol}$  creatinine) and England (194 (SD 78)  $\mu\text{mol}/\text{mmol}$  creatinine). The excretion of 5-L-oxoproline in infants in Trinidad was similar for each of the three groups. There were no differences in urinary 5-L-oxoproline in relation to sex. A reliable feeding history was obtained from forty-one infants from Trinidad; nine were consuming only breast milk, five were consuming an infant formula, and twenty-seven were receiving a mixture of breast and formula feeding. For twenty infants in England, fourteen were consuming breast milk only, three were receiving infant formula, and three were receiving both breast milk and formula. As shown in Fig. 1, there was no difference in urinary 5-L-oxoproline in relation to the pattern of feeding in either site, and for every pattern of feeding the urinary excretion of 5-L-oxoproline was greater in Trinidad than in England.

Using simple correlation analysis for all the infants, there was a significant relationship between maternal haemoglobin and head circumference at birth ( $r = -0.28$ ,  $P = 0.03$ ), which was much stronger when the infants in Trinidad were considered alone ( $r = -0.44$ ,  $P = 0.008$ ). The correlation between maternal haemoglobin and birth weight failed to reach conventional statistical significance ( $r = -0.22$ ,  $P = 0.09$ ). There was a significant correlation between current head circumference and 5-L-oxoproline/creatinine ( $r = -0.29$ ,  $P = 0.024$ ) with weaker correlations for 5-L-oxoproline/creatinine and birth weight ( $r = -0.23$ ,  $P = 0.05$ ), and head circumference at birth ( $r = -0.20$ ,  $P = 0.12$ ). When the effect of maternal haemoglobin was taken into account in a partial correlation analysis, there were significant relationships between 5-L-oxoproline/creatinine and current head circumference ( $r = -0.36$ ,  $P = 0.008$ ), birth weight ( $r = -0.32$ ,  $P = 0.028$ ), and head circumference at birth ( $r = -0.27$ ,  $P = 0.05$ ). For each of these the correlation was inverse, indicating that higher levels of 5-L-oxoproline excretion in urine were associated with smaller size. There was a significant relationship ( $R^2 = 0.18$ ;  $F = 3.7$ ;  $P = 0.017$ ), when 5-L-oxoproline was the dependent variable in a multiple linear regression analysis which included current



**Fig. 1.** The relationship between pattern of feeding and urinary excretion of 5-L-oxoproline in a single sample of urine collected from infants about 6 weeks of age, in Trinidad ( $n = 41$ , □) or in England ( $n = 20$ , ▨). Urinary excretion was significantly different in Trinidad compared with England for breast fed ( $*P = 0.02$ ), formula fed ( $**P = 0.025$ ) and breast plus formula fed infants ( $***P = 0.077$ ) using the Kruskal-Wallis test.

head circumference ( $P = 0.0076$ ), maternal haemoglobin ( $P = 0.083$ ) and change in weight from birth ( $P = 0.096$ ).

## Discussion

In a previous study we found that the excretion of 5-L-oxoproline in infants in Jamaica at 4 weeks of age was 300  $\mu\text{mol}/\text{mmol}$  creatinine, and at 6 weeks of age it was 500  $\mu\text{mol}/\text{mmol}$  creatinine (Persaud *et al.* 1997), compared with an excretion of about 200  $\mu\text{mol}/\text{mmol}$  creatinine in infants in England (Jackson *et al.* 1997a). In the present study we have shown that the excretion of 5-L-oxoproline in infants between 4 and 6 weeks of age in Trinidad, about 400  $\mu\text{mol}/\text{mmol}$  creatinine, was very similar to the excretion in infants in Jamaica and about twice that measured in infants in England. Further, there were no differences in the excretion of 5-L-oxoproline amongst the three groups in Trinidad, infants of Indian, African or mixed parentage. Nor were there any differences in relation to the pattern of feeding, as for both Trinidad and England excretion was similar in infants who had only ever received breast milk, were receiving an infant formula, or who were receiving breast milk together with an infant formula. In the present study we had the opportunity to explore the relationships amongst maternal factors and fetal growth with the excretion of 5-L-oxoproline at later ages. The mothers from Trinidad and England were similar in many respects, being of similar age and parity and having comparable levels of haemoglobin during pregnancy. There were, however, significant differences in the size at birth and growth of the infant. Care has to be used in the interpretation of birth size unless the duration of pregnancy has been carefully timed. Nevertheless, both head circumference and weight at birth were significantly

less in Trinidad than England. By the time of study the differences in weight were no longer evident, but the differences in head circumference persisted. These data provide clear evidence of diminution in growth and relative disproportions in growth for the infants in Trinidad compared with the infants in England.

Maternal haemoglobin has been used as an index of maternal nutritional status (Garn *et al.* 1981). Maternal haemoglobin has been related to disproportions in placento-fetal growth and the risk of the development of high blood pressure at later ages (Godfrey *et al.* 1991). In Jamaica there was an indirect relationship between maternal haemoglobin and blood pressure in children at 11 years of age, with maternal adiposity also playing a part in the relationship (Godfrey *et al.* 1994). In the present study there was an association between fetal growth, head circumference at birth and birth weight, and maternal haemoglobin taken on a single occasion late in pregnancy. When multiple regression analysis was used to explore the factors which might contribute to explaining the variability in urinary 5-L-oxoproline, birth weight, head circumference at birth and the time of study and maternal haemoglobin all made a contribution. However, the relative contribution of each of these factors was modest when compared with the powerful effect exerted by place of birth on urinary 5-L-oxoproline.

The results of the present and the previous study (Persaud *et al.* 1997) show clearly that the urinary excretion of 5-L-oxoproline for infants at about 6 weeks of age in the Caribbean is twice that for infants of similar age in England (Jackson *et al.* 1997a). As a further step in exploring whether any of the difference might be explained by ethnic factors we have measured urinary 5-L-oxoproline in ten infants of Indian descent born in England. The birth weight of these infants was 3.11 (SD 0.29) kg and at 6 weeks of age urinary 5-L-oxoproline was 155  $\mu\text{mol}/\text{mmol}$  creatinine, significantly lower than any group of infants from Trinidad ( $P < 0.001$ ), but not different to infants in England of Caucasian descent. Therefore the differences in the urinary excretion of 5-L-oxoproline amongst infants in England and the Caribbean can not be attributed to ethnic differences. Although size at birth and growth after birth may make an important contribution to the differences, the overwhelming influence is environmental.

Urinary 5-L-oxoproline can be used as a marker for glycine status (Jackson *et al.* 1987) which, although the simplest amino acid, plays a central role in metabolism (Jackson, 1991). Its disproportionate requirement in the formation of collagen means that the need is particularly increased during periods of bone formation and growth. Cellular multiplication utilizes glycine for DNA and RNA synthesis and haem formation, hepatic excretory capacity and the formation of bile salts impact upon a wide range of fundamental metabolic processes. Given the likelihood that the difference we have found between infants in the Caribbean and in England is due to environmental factors, rather than genetic or ethnic differences, one important possibility is the extent to which the ability to form glycine endogenously is itself a function which is programmed by intra-uterine factors. Were this to be so, it would help to explain a link between fetal and infant growth and later

reproductive competence of the mother (Lumey, 1992). If the availability of glycine were limiting for fetal growth in the Caribbean, this might help explain the predisposition to heart disease and diabetes in the Indian population (Miller *et al.* 1982) and hypertension, stroke and diabetes in the African population (Miller *et al.* 1988, 1989; Wilks *et al.* 1996).

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