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GROWTH AND METABOLISM IN HOMOZYGOUS SICKLE CELL DISEASE

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**A Child with Growth Retardation, Hypersplenism and
Homozygous Sickle Cell Disease.**

ABSTRACT

Growth impairment in homozygous sickle cell (SS) disease emerges as early as age six months but the mechanisms for this deficit are unknown. Analysis of longitudinal growth data suggested that adolescent growth and pubertal maturation of Jamaican SS children was delayed but final height was normal. This observation, delayed skeletal maturation, reduced weight for height, and lower subcutaneous fat reserves are consistent with the hypothesis that chronic childhood malnutrition retards growth in SS children.

Competition from erythropoiesis may limit the availability of nutrients for growth and this hypothesis was supported by observations that high fetal haemoglobin levels, which reduce the haemolytic rate and therefore erythropoiesis, were associated with more normal growth, and that reducing erythropoiesis in SS patients with chronic hypersplenism by splenectomy was followed by an acceleration in linear growth. High erythropoietic activity may increase metabolism and the mean resting metabolic rate (RMR) relative to lean body mass of 16 post-pubertal SS adolescent boys was 22% greater than age and sex matched controls with a normal (AA) haemoglobin genotype. Adjusting for the higher visceral to somatic mass ratio of SS boys reduced the increase in RMR in SS disease to 8%. The RMR of prepubertal SS boys was also increased (relative to predicted values) but this increase did not correlate significantly with growth or serum transferrin receptor concentrations (a measure of erythropoietic activity).

Faced with higher metabolic demands, SS patients have the option of increasing calorie intake or reducing energy expenditure for physical activity. The physical activity level of SS adolescent boys was 30% lower than age and sex matched AA controls suggesting that either the availability of calories or a poor appetite prevented an energy intake sufficient to maintain physical activity. It is therefore postulated that correction of suboptimal childhood nutrition could benefit the physical and mental development of children with SS disease.

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PREFACE

The substitution of valine for glutamic acid on the beta chain of the haemoglobin molecule and the subsequent tendency for haemoglobin S molecules to form polymers has affected the lives of many thousands of the worlds people. Sickle cell disease is the most common genetic condition to affect Afro-Caribbean populations but despite an improvement in mortality, a high morbidity in early childhood continues to have a devastating effect on patients and their families.

Poor growth and development and consequently psychological problems during adolescence are common sources of childhood morbidity. Deficits in height and weight emerge in SS children from as early as 6 months of age but the aetiology of retarded growth is unknown. Mechanisms for growth impairment in SS disease are probably multifactorial with contributions from abnormal endocrine function, sub-optimal nutrition, and increased metabolism secondary to the high rate of erythropoiesis. This thesis aims to investigate the determinants of growth retardation in SS disease and to assess the hypothesis that suboptimal childhood nutrition secondary to an increase in metabolic activity retards growth of clinically *vague* asymptomatic SS children.

This work specifically aims to:

1) use longitudinal data from the Jamaican cohort study to assess the overall pattern of growth in SS disease (including adolescent growth and pubertal development).

2) to examine the variability in growth and to investigate possible determinants of poor growth.

3) to assess the possible contribution of increased erythropoietic activity to growth impairment by using SS patients with hypersplenism as a model of extreme erythropoietic stress.

4) to consider the hypothesis that competing demands from erythropoiesis *(or anaemia per se?)* limit nutrient and/or energy availability for growth.

5) to investigate the possible effect of increased erythropoietic activity on resting metabolic rate in SS children.

6) to elucidate the determinants of the high resting metabolic rate in asymptomatic (steady-state) patients with SS disease particularly in relation to possible genotype differences in body composition.

7) to investigate the consequences of a high resting metabolic rate on energy balance and to assess possible factors affecting energy balance in steady-state SS children.

The concepts of sickling, vaso-occlusion and haemolysis are central to the pathophysiology of homozygous sickle cell disease, but the possibility of covert metabolic problems in asymptomatic SS patients has received relatively little attention. The metabolic cost of the increase in haemolysis and erythropoiesis and their effects on growth, disease severity and nutritional status in SS disease has been inadequately studied and the present work furthers knowledge in these areas.

Acknowledgements

Principle Symbols and Abbreviations

AA	Normal haemoglobin genotype
BMI	Body mass index (weight/height^2)
CI	Confidence interval
CO_2	Carbon dioxide
CRP	C-reactive protein
FSH	Follicle stimulating hormone
GnRH	Gonadotrophin releasing hormone
Hb	Haemoglobin
HbF	Fetal haemoglobin
HR	Heart rate
LBM	Lean body mass
LH	Luteinising hormone
MCH	Mean cell haemoglobin
MCHC	Mean cell haemoglobin concentration
MCV	Mean cell volume
$MJ.kg^{-1}.d^{-1}$	Mega Joules per kilogram body weight per day
NCHS	National Centre for Health Statistics
O_2	Oxygen
PAL	Physical activity level
RBC	Red blood cell count
PHV	Age at peak height velocity of the adolescent growth spurt
RMR	Resting metabolic rate
RQ	Respiratory quotient
SAA	Serum amyloid A protein
SC	Sickle cell-haemoglobin C disease
SD	Standard deviation
SES	Socioeconomic score
SS	Homozygous sickle cell disease
TBW	Total body water
TDEE	Total daily energy expenditure
TfR	Transferrin receptor
TO	Age at onset of the adolescent growth spurt (Take off)
VO_2	Oxygen consumption
z	Standardised score

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CHAPTER I: INTRODUCTION AND BACKGROUND

PHYSICAL AND SEXUAL DEVELOPMENT

In the first description of a female patient with homozygous sickle cell (SS) disease, Washburn (1911) noted a delay in age at menarche to 18 years. Mason (1922), and later Diggs and Ching (1934) recorded a slender build and poorly developed sexual characteristics in SS adults. Since these reports, impaired growth, delayed puberty, and abnormal body habitus have been extensively documented in SS disease, although the absence of longitudinal data has prevented analysis of the overall pattern of growth.

Prepubertal Growth

Small numbers of children (Winsor and Burch, 1944) or a lack of racially appropriate control observations (Scott 1955) make early studies of preadolescent growth in SS disease difficult to interpret. In one of the first controlled studies, Whitten (1961) found significantly lower heights and weights in 48 children with SS disease (aged 2-13 years) compared to sibling controls. Relative to normal white American children, the mean percentiles for height (25.5) and for weight (17.1) in children with SS disease were less than in black control subjects (39.7 and 41.4 respectively), and suggested a greater deficit in weight than height.

Two other studies from the United States reported both subnormal height and weight in children with SS disease (Jimenez *et al*, 1966; McCormack *et al*, 1976). McCormack *et al* also found lower upper arm circumference, calf circumference and sitting heights in patients compared to controls, and boys to be thinner (based on $\log_{10}$ weight for height indices and triceps thickness) than girls with SS disease.

In contrast to American reports, Jamaican children with SS disease manifested subnormal weight but not height. The mean height of 100 SS children (aged 1-12 years, Gray *et al*, 1971) followed the 50th centile of Jamaican growth standards of children with a normal (AA) haemoglobin genotype (Ashcroft and

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Lovell, 1966) but weight fell away after the age of 8 years. Reporting cross sectional data from 99 Jamaican children aged 2-13 years, Lowry *et al* (1977) also noted the weight of Jamaican SS children to be consistently below the same local standards, the difference becoming statistically significant after age 10 years. Little difference in height was observed possibly because SS children detected by family studies are likely to manifest less severe clinical disease and therefore have better linear growth than symptomatically selected American populations.

Data from Africa (Lesi 1979) and the United Kingdom (Mann 1981) are consistent with greater deficits in weight than height in prepubertal children with SS disease, although these studies can both be criticised for the use of white standards for comparison. African children aged 1-10 years of low socioeconomic status had subnormal height and weight but children of a high socioeconomic background manifested only a height deficit (Lesi 1979).

Ascertainment biases arising from symptomatically selected patients make early reports of growth in SS disease difficult to interpret. A cross sectional study design and the lack of data from children less than 1 year of age prevents assessment of the timing of growth impairment and introduces bias as more severely affected children are likely to succumb to the high early disease mortality. To address these criticisms Kramer *et al* (1980) reported the first longitudinal growth data from children with SS disease detected by cord blood screening and AA controls matched for age, sex and socioeconomic status. Birth weights of the 11 SS children in this study were normal (as previously reported, Booker *et al*, 1964), but significant deficits in height, weight and total skinfold thickness (triceps + subscapular) emerged from as early as age six months.

A study of 298 SS children and 231 AA controls diagnosed and followed from birth (Stevens *et al*, 1986) provided the first longitudinal growth data from Jamaican SS children. Statistically significant deficits in height and weight of patients were noted before 2 years of age and although there were only small numbers of subjects at older ages (29 boys and 30 girls at age nine years), the

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growth impairment in SS disease appeared to be progressive, with height falling to 1 standard deviation below the mean for AA controls by age nine years. Detailed anthropometric examination of 123 SS children aged 4-6 years from the same cohort also showed a reduction in inter-acromial and inter-cristal diameters, and skinfold thicknesses (Stevens *et al*, 1983), consistent with data from McCormack *et al* (1976). However, in contrast to McCormack's study, Jamaican boys had better growth than girls with SS disease.

Adolescent Growth

In the first anthropometric studies, Winsor and Burch (1944) concluded that adults, adolescents and children with SS disease tended to have a slender build, longer legs, and a shorter trunk than haematologically normal American blacks. Later reports by Whitten (1961) and Jimenez *et al* (1966) also observed subnormal weight and height in SS adolescents.

Growth data from Jamaican adolescents with SS disease were first made available by Ashcroft *et al* (1972) who studied a cross-sectional sample of 99 SS adolescents aged 12-21 years and local control subjects of a similar age. Weight related to height, chronological or bone age tended to be lower in patients than in controls. Heights were also lower but showed progressively less deficit with age and suggested catch up in height during adolescence, an hypothesis supported by similar adult heights in Jamaican SS patients and controls (Ashcroft and Serjeant, 1972). Reports from Africa (Olambiwonnu *et al*, 1975) and America (McCormack *et al*, 1976), however, failed to confirm a decreasing height deficit during adolescence possibly because of the small numbers of patients over 15 years of age in both studies. Furthermore, in contrast to Jamaican patients, Phebus *et al* (1984) reported an increasing height deficit with age in 133 American children with SS disease aged 1 to 18 years.

Cross-sectional data from 1404 American patients confirmed height and weight deficits in adolescents with SS disease (Platt *et al*, 1984). Again differences

in weight were more pronounced than those in height which tended to be small in adults.

The first longitudinal study of adolescent growth in SS disease was based on a three year follow up of 55 American children (26 boys and 29 girls) aged 13-18 years (Luban et al, 1982). Weight and height of patients were reported to be below National Center for Health Statistics (NCHS) standards (Hamill et al, 1979), but skinfold thicknesses were all within normal limits. However, in this preliminary report, the number of children at each age was not given and growth data were not reported.

Serial growth observations have also been reported from a small number of American SS children (7 boys and 6 girls) passing through the adolescent growth spurt (Phebus et al, 1984). Compared to NCHS standards, the age at peak height velocity in boys with SS disease (16.3 years) was delayed by more than 2 standard deviations from the mean of normal boys. Pubertal delay appeared to be less marked in SS girls, the mean age at peak height velocity (14.4 years) being delayed by 2.4 years but falling within 2 standard deviations of the mean for normal girls.

Body Habitus

A slender body habitus, long limbs and reduced body weight is characteristic of patients with SS disease. Differences in body shape, including narrower pelvic and pectoral girdles and an increase in anterior-posterior relative to lateral chest diameter (Ashcroft and Serjeant, 1972; McCormack et al, 1976; Stevens et al, 1983) are apparent in children as young as 6 years of age (Stevens et al, 1983). Anthropometric assessment of the adult habitus also consistently indicates a low sitting height to standing height ratio (Winsor and Burch, 1944, 1945; Ashcroft and Serjeant, 1972) secondary to both reduced sitting height (McCormack et al, 1976; Ashcroft and Serjeant, 1972) and increased leg length (Ashcroft and Serjeant, 1972). Children with SS disease, however, have a normal sitting to standing height ratio (Whitten 1961; Stevens et al, 1983) and shorter limbs

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(Stevens *et al*, 1983), suggesting that genotype differences in this index of body shape in adults emerge during adolescence. Whitten (1961) was the first to suggest that abnormal adult habitus may result from delayed epiphyseal fusion allowing more time for longitudinal bone growth, an hypothesis supported by his observation of delayed skeletal maturation in SS children aged over 11 years.

Skeletal Development

Whitten (1961) observed a delay in skeletal maturation in 7/30 adolescents with SS disease, Watson-Williams (1962) described a 27 year old Nigerian male with a bone age of 9.5 years, and Danowski *et al* (1965) a 17 year old male with a bone age of 12 years. The first survey of skeletal development in SS disease (Ashcroft and Serjeant, 1972) noted the bone ages of 12-21 year old SS patients (estimated by the method of Greulich and Pyle) to be less than that of AA controls at all ages. A delay in skeletal maturation also occurred in all 81 Jamaican adolescents aged 12-19 years with unfused epiphysis (Serjeant and Ashcroft, 1973) and in 13/15 African children with SS disease aged 5-16 years (Olambiwonnu *et al*, 1975).

There is general agreement that skeletal maturation is retarded in SS disease but the age at which the delay emerges is uncertain. Retarded skeletal maturation was noted at all ages in 46 American SS children aged 1-17 years (McCormack *et al*, 1976) but the delay was not quantified. Skeletal retardation from as young as age 3 years was also observed in 80 American children with SS disease (Harris *et al*, 1976), and, in a Jamaican survey of 120 SS children (Lowry *et al*, 1978), from 8 years in boys and from as early as 2 years in girls. The sex difference was not explained.

Subsequent prospective studies sought to identify the onset of skeletal retardation in SS disease. Longitudinal observations in American SS children suggested a delay in skeletal maturation by age 3-6 years (Kramer *et al*, 1980), although the extent of impairment was not quantified. Compared to AA controls, delays in skeletal maturation of 0.4 years in boys and 0.6 years in girls occurred in

Jamaican SS children by age 5 years (Stevens *et al*, 1986). The genotype difference at age 5 years was not statistically significant but increased to 1 year in boys and 1.3 years in girls by age 8, reaching statistical significance and suggesting a progressive impairment of skeletal maturation.

Sexual Development

In the earliest reference to sexual development in SS disease, Anderson and Ware (1932) noted infantile genitalia in 26% of patients aged over 10 years. Sharp and Vonder Heide (1944) observed a eunuchoid habitus in SS adults and Jimenez *et al*, (1966) and Olambiwonnu *et al*, (1975) reported a delay in development of secondary sexual characteristics in both sexes. Luban *et al* (1982) also commented that sexual development was delayed in 13-18 year old adolescents with SS disease, but development progressed in an 'orderly' fashion. In all of these studies the small numbers of SS children and lack of appropriate control subjects prevented the degree of delay in sexual maturation from being quantified.

The Co-operative Study (Platt *et al*, 1984) attempted to quantify the genotype difference in sexual maturation by comparing the sexual development of SS children with data from normal children suitable for reference, but not statistical comparison. The age at development of all Tanner stages in SS children was retarded compared to normal children and to subjects with sickle cell-haemoglobin C (SC) disease or sickle cell-B^+ thalassaemia. The median age at Tanner stage V breast development, 17.3 years for SS, 17.2 for sickle cell-B^0 thalassaemia, 16.5 for sickle cell-B^+ thalassaemia and 16.0 years for SC subjects, indicated the degree of retardation.

A delay in menarche is consistent with the general delay in adolescent growth and development in SS disease. Jimenez *et al*, (1966) reported a mean age at menarche of 13.9 years in American girls with SS disease compared to 12.2 years in black controls of unspecified genotype. The mean age at menarche in Jamaican SS girls was reported as 15.4 (SD: 1.7) years (Alleyne *et al*, 1981), 15.7

(2.0) years (Ashcroft and Serjeant, 1981), and 16.1 (1.9) years (Graham *et al*, 1986) compared to 13.1 (1.7) years in AA controls (Alleyne *et al*, 1981). However, these studies can all be criticised for relying on patient recall, a technique sensitive to errors of recall and of bias in any series that includes girls who have not menstruated (Eveleth and Tanner, 1976). In contrast, in the Co-operative Study (Platt *et al*, 1984) girls aged over 10 years were asked whether they had ever had a period and, using this 'status quo' technique, menarche was reported to be 1-2 years later in SS than SC girls. Menarche data for AA girls were not available in the Co-operative study.

Summary

Retarded growth is common in prepubertal children with SS disease. A progressive deficit in height and weight appears to emerge at around 6 months of age and skeletal maturation is retarded from as early as age 5 years (Stevens *et al*, 1986). Weight appears to be impaired more than height (Luban *et al*, 1982; Platt *et al*, 1984) and boys may be more severely affected than girls (McCormack *et al*, 1976; Phebus *et al*, 1984). Patients may also have differences in body shape, thinner skinfolds and reduced mid-arm circumference (McCormack *et al*, 1976; Stevens *et al*, 1983) compared to AA controls.

Adolescent growth and pubertal development are delayed in SS disease but a normal final height (Ashcroft and Serjeant, 1972; Platt *et al*, 1984) and orderly progression of sexual maturation (Luban *et al*, 1982) suggest a constitutional retardation of adolescent growth. The delay in epiphyseal closure, which tends to be more marked in older children (Serjeant and Ashcroft, 1973), is consistent with this hypothesis and may allow a longer period for longitudinal bone growth, catch up in final height and reduction in the sitting height to standing height ratio. However, analysis of adolescent growth abnormalities has been limited by the lack of longitudinal observations in individuals and by an inability to quantitate the observed patterns.

DETERMINANTS OF ABNORMAL GROWTH

Determinants of abnormal growth and sexual development in SS disease are likely to include haematological, social and nutritional factors. Endocrine abnormalities and chronic disease may influence growth in children with a normal haemoglobin genotype but their effect in SS disease requires clarification.

Haematology

Whitten (1961) first assessed the effect of abnormal haematology on growth in SS disease when he reported that attained height was not related to haemoglobin (Hb) concentration or degree of haemoglobin oxygen saturation although maintaining a Hb concentration above 90 g/L by multiple transfusions was associated with a marked increase in weight in one of two children.

The effect of chronic anaemia on growth in SS disease is difficult to quantitate since steady-state Hb levels vary widely between individuals and the determinants of the Hb level are poorly understood. Haemoglobin concentration is only one of several factors determining oxygen delivery in SS disease (Milner 1974), the simple equation between Hb concentration and oxygen carrying capacity being offset by the lowered affinity of HbS, the marked variability in the degree of shift in the oxygen dissociation curve between patients and the inverse relationship between P_{50} and the Hb level. This implies that high Hb levels tend to be associated with high oxygen affinity and vice versa thus tending to minimise any relationships between Hb concentration and oxygen delivery (Serjeant *et al*, 1996). Furthermore as a consequence of excessive plasma volume expansion, the Hb concentration also underestimates the red cell mass (Steinberg *et al*, 1977). It is not surprising therefore that most studies have failed to find a consistent relationship between Hb level and growth (Ashcroft and Serjeant, 1972; Serjeant and Ashcroft, 1973; Lowry *et al*, 1977).

Haemoglobin F levels

It is widely accepted that high haemoglobin F (HbF) levels in SS disease are associated with a less severe clinical and haematological course (Serjeant 1975; Bailey *et al*, 1992). Differences in HbF levels could therefore contribute to the marked variability in growth of SS children and Serjeant and Ashcroft (1973) reported significant correlations of low HbF with a greater delay in skeletal maturation in children of both sexes. Delayed epiphyseal fusion allowing a longer period for leg bone growth (so increasing leg length relative to spinal length) could also explain the low sitting to standing height ratio in adult men with low HbF (Ashcroft and Serjeant, 1972). The mechanism for this delay is unknown, but a symmetrical and generalised delay in skeletal maturation argues against the possible effects of epiphyseal damage from random and local vaso-occlusion. The correlation of HbF levels with skeletal maturation in post-pubertal but not prepubertal SS children (Stevens *et al*, 1986) suggests that this relationship emerges during adolescence and may be secondary to a general delay in adolescent growth in SS children with low HbF. Significantly greater attained weight in 10-13 year old children with high HbF levels (Lowry *et al*, 1977) were consistent with this hypothesis, although associations between HbF and growth indices in most studies have always been weak possibly because patients with high HbF levels and mild disease are under-represented in symptomatically selected populations.

Socioeconomic Status

Social class affects growth and development in children with a normal AA haemoglobin genotype (Martorell 1985) and those with SS disease (Lesi 1979; Ashcroft and Serjeant, 1981). The Co-operative Study of sickle cell disease in the USA failed to find a relationship between family income and height, weight, or age at menarche in SS disease (Platt *et al*, 1984) possibly because children of a high social class and mild disease were likely to be under-represented in this

symptomatically selected population. In Jamaica the age of menarche correlated negatively with socioeconomic status (Alleyne et al, 1981; Ashcroft and Serjeant, 1981; Graham et al, 1986) and there is a strong clinical impression, although few data, suggesting that social class also affects linear growth in SS disease.

Disease Severity

The effect of disease severity on growth has not been systematically studied. Whitten (1961) compared disease severity (estimated retrospectively from patient records) in SS children of low or normal weight and found no difference in the frequency of clinical crises, respiratory tract infections or 'haematological' crises. Abnormal body habitus in SS adults was associated with the presence of leg ulceration (Ashcroft and Serjeant, 1972) but this relationship is likely to be secondary to low HbF being a risk factor for both conditions. Lowry et al (1977) also found no significant association with disease severity (estimated by the number of hospital admissions) and the heights and weights of Jamaican children with SS disease although difficulties in assessing disease severity from retrospectively collected data seriously limits the contribution of this and previous studies.

Endocrinology

Hypogonadism

Delayed endocrine maturation may retard physical and sexual development. Gonadal function is impaired in some patients (Parshad et al, 1994) but the contribution of hypogonadism to retarded growth and development in children with SS disease has not been established.

Testosterone levels are low in adolescent males with SS disease (Odonkor and Addae, 1982; Landefeld et al, 1983), although the lack of normal AA controls matched for the stage of puberty makes these studies difficult to interpret. Most, but not all, studies (Friedman et al, 1974) also report low testosterone levels in SS adults (Abbasi et al, 1976; Dada and Nduka, 1980; Odonkor and Addae, 1982; Odonkor 1983) and there is increasing evidence for primary testicular failure as the

most likely mechanism for this deficiency. High levels of luteinizing hormone (LH) and follicle stimulating hormone (FSH) (Olambiwonnu *et al*, 1975; Abbasi *et al*, 1976) secondary to reduced negative feedback to the hypothalamic pituitary axis and the tendency for higher gonadotrophin levels to be associated with lower testosterone levels (Osegbe and Akinyanju, 1987) are consistent with this hypothesis. Small testicular volumes that correlate with low serum testosterone levels (Abbasi *et al*, 1976), histological evidence of relative immaturity of seminiferous tubules with arrest or absence of spermatozoa (Jimenez *et al*, 1966; Abbasi *et al*, 1976) and reduction in sperm volume, count and motility (Friedman *et al*, 1974; Osegbe *et al*, 1981) all support poor testicular function in SS adults. Compared to normal controls matched for age and pubertal stage, LH levels were high in SS children less than 10 years of age but normal in older patients suggesting a transient impairment of gonadal function in SS disease (Olambiwonnu *et al*, 1975). Careful matching of SS and AA control subjects by pubertal stage is essential and lack of such matching in some studies could account for reports of 'normal' gonadotrophin levels in SS disease (Dada and Nduka, 1980; Landefeld *et al*, 1983; Osegbe and Akinyanju, 1987).

Stimulation of gonadotrophins by Gonadotrophin Releasing Hormone (GnRH) has not clarified the mechanism of low testosterone levels in SS disease. Abbasi *et al*, (1976) noted an exaggerated response to GnRH of both FSH and LH, consistent with a primary testicular failure, but Parshad (1994) found the proportionate increase in gonadotrophin level over baseline to be similar in 10 SS adult males and their age matched AA controls. In contrast, Landefeld *et al*, (1983) reported a reduced gonadotrophic response in 2 SS patients with hypogonadism suggesting a partial hypogonadotrophic hypogonadism. Treatment with the GnRH stimulator, Clomiphene, was followed by a rapid onset of puberty and increased gonadotrophin and testosterone levels, suggesting that the pituitary-hypothalamic axis was mature but insufficient endogenous GnRH release had reduced the responsiveness of the pituitary to GnRH. The authors suggested that

their data were consistent with hypothalamo-pituitary abnormalities in their 2 patients but extrapolation of these findings to the general SS population is not justified.

Growth Hormone

Despite the possible contribution of growth hormone deficiency to growth failure, few studies have investigated growth hormone levels in SS disease. Odonkor *et al*, (1983b) reported lower growth hormone levels in nine SS adolescents aged 14-16 years compared to 5 age matched AA controls, and higher levels in 13 SS adults (aged 18-25 years) compared to 6 age matched controls. Patients and controls, however, were not matched for the stage of puberty and as growth hormone levels rise during the adolescent growth spurt, the later physical development of SS adolescents may explain these results. Growth hormone levels following an extended glucose tolerance test were also normal but standard growth hormone stimulation tests (such as with insulin or clonidine) have not been used to assess pituitary growth hormone reserve in SS children.

Leiba *et al*, (personal communication) explored the relationship of height velocity and bone age with serum insulin-like growth factors (IGF), the mediators of the growth promoting effects of growth hormone. IGF-1 levels were significantly lower in SS compared to age and sex matched AA subjects from as young as 5 years of age but did not correlate with weight and height velocity in either genotype. The age related increase in IGF-1 between 5 and 12 years was also greater in AA than SS subjects and as IGF-1 levels are influenced by nutritional status (Unterman *et al*, 1985) these results were consistent with relatively poor nutrition in SS children. However longitudinal studies are needed to clarify the relative contribution of abnormal endocrinology and inadequate nutrition to low IGF-1 levels and retarded growth in SS patients.

Thyroid Function

Levels of tri-iodothyronine (T3) and thyroxine (T4) were normal in 2 African studies; Odonkor *et al*, (1982a) reporting a small group of SS adolescents aged 14-

16 years and Lukanmbi *et al* (1986) children aged 1-15 years. In Jamaica, tri-iodothyronine levels were significantly lower, thyrotropin (TSH) higher, and the response to thyrotropin releasing hormone greater in SS compared to AA adult males (Parshad *et al*, 1989). These findings are consistent with a mild primary thyroid failure, although a low body weight, an elevated basal metabolic rate (Odonkor *et al*, 1982a) and lack of clinical evidence of hypothyroidism in SS disease appear inconsistent with this hypothesis.

Summary

Growth retardation in SS disease is likely to be multifactorial with contributions from abnormal haematology and endocrine function. However, previous studies based on small numbers of symptomatically selected patients have identified few determinants of abnormal growth. Low HbF levels may correlate with a delay in skeletal maturation but there is little evidence to suggest that Hb concentration influences linear growth. Endocrine studies are consistent with a primary testicular failure, but the pathogenesis and effect of low testosterone levels on growth in SS disease are not clear. The influence of socioeconomic status and disease severity on growth has also not been systematically investigated.

Low weight, reduced height velocity for age, reduced skinfold thickness, and a delay in menarche and puberty are consistent with a role for suboptimal childhood nutrition in the growth retardation of SS children.

NUTRITIONAL FACTORS IN GROWTH RETARDATION

Many studies from the developed and developing worlds have emphasised the importance of adequate nutrition for normal growth and development of children (Martorell 1985). The influence of nutrition on the growth of children with sickle cell disease, however, is poorly understood since the interpretation of most studies is limited by small sample size, inadequately matched control subjects and cross-sectional study design. Poor growth in SS children has been attributed to a deficiency of micronutrients such as zinc or folic acid and only recently have macronutrients such as protein and energy been investigated. The high metabolic demands of an expanded bone marrow have also recently drawn attention.

Pathogenesis of Nutrient Deficiencies

Decreased intake, intestinal malabsorption, and increased catabolism of specific nutrients may lead to nutrient deficiencies in SS disease. Although most available data suggest similar food intake and absorption in SS and AA subjects, nutrient intake may be insufficient to meet higher metabolic demands in SS patients.

Increased Metabolic Demands

Marked erythrocyte destruction is central to the pathology of SS disease. Estimated red cell life span is 10-12 days in patients compared to 120 days in normal subjects (London *et al*, 1949) and in the face of rates of haemolysis as high as 20 times normal (Bensinger and Gillette, 1974) haemoglobin levels can only be maintained by a marked erythropoietic expansion. Serjeant (1974) estimated that haemoglobin synthesis is increased from 6.25 g/day in normal adults to 40 g/day in patients, leading to an increase in demands for protein, energy, iron and folate. Haemolysis is particularly severe in patients with chronic hypersplenism and preliminary observations suggest that the metabolic cost of erythropoiesis in these patients may be higher than in patients without this complication.

Hypersplenism and Growth

Splenic enlargement in SS disease characteristically occurs in the first year of life and is usually followed by a progressive splenic fibrosis generally attributed to the development of intravascular sickling. This natural history may be punctuated in some patients by episodes of acute splenic enlargement with profound haematological consequences (acute splenic sequestration) or by sustained splenic enlargement with chronic sequestration and marked haematological abnormalities (chronic hypersplenism). Mean red cell life spans as short as 2 days have been recorded in patients with chronic hypersplenism and the erythropoietic expansion required to maintain haemoglobin levels of 30-60 g/L is likely to have a high metabolic cost. The fall in protein turnover (Badaloo *et al*, 1991) and preliminary observations of a growth spurt following splenectomy for chronic hypersplenism (Emond 1987) are consistent with this hypothesis. The factors influencing the catch up in growth after splenectomy are poorly understood but an improvement in red cell survival (Emond *et al*, 1984) and fall in protein turnover (Badaloo *et al*, 1991) suggest that a high metabolic load competes with the requirements for normal growth and is relieved by splenectomy.

Intestinal Function

Reports of high faecal nitrogen losses (Odonkor *et al*, 1984) and reduced D-xylose absorption in children (Grange 1982) suggest that impaired gastro-intestinal function may contribute to growth retardation in SS disease. Lower serum xylose levels 1 or 2 hours after ingestion in SS compared to AA adults, were consistent with impaired carbohydrate absorption (Odonkor *et al*, 1983a), although the lack of a genotype difference in the 5 hour urinary excretion of xylose, is consistent with enhanced renal clearance of xylose (secondary to the high glomerular filtration rate in SS disease) being the underlying basis for this observation.

In contrast, Rahbar *et al*, (1977) found intestinal absorptive function to be normal in 16 children with SS disease compared to 18 non-anaemic controls. Although SS patients may have low serum lipid concentrations (Muskiet and

Muskiet, 1984), fat absorption (measured as fat intake minus fat excretion, relative to intake) was normal in 5 growth retarded SS boys (Heyman *et al*, 1985). Furthermore, normal intestinal biopsies obtained in patients from 3 studies argues against significant intestinal pathology in SS disease (Rahbar *et al*, 1977; Heyman *et al*, 1985, 1989).

Only one study assessed intestinal function in SS disease, Heyman *et al*, (1989) reporting elevated breath hydrogen gas concentrations in 8 fasting SS children compared to previously published values from children with normal haemoglobin. The rise in breath hydrogen (produced by intestinal bacterial metabolism after ingestion of the non-absorbable carbohydrate, lactulose) was also greater in SS subjects compared to published values in normal children. Although intestinal cultures were not obtained, these results were consistent with increased hydrogen production from small intestinal bacterial overgrowth in SS disease. Interestingly, there was an earlier rise in breath hydrogen production after carbohydrate ingestion in the 4 growth retarded (weight and height for age below the 5th percentile for NCHS reference centiles) compared to the 4 normally growing SS children, suggesting an association between rapid intestinal transit time and growth retardation in SS disease.

Micronutrient Deficiencies

Zinc

The role of zinc deficiency in the growth retardation of SS disease has been extensively investigated, but most studies are small and the data often conflicting. Compared to normal controls, several authors report low plasma zinc levels in SS disease (Serjeant *et al*, 1970; Prasad *et al*, 1975, 1976; Niell *et al*, 1979), although normal levels have also been reported in a small Nigerian study of only 13 SS adults (Kapu *et al*, 1976). However, since haemolysis raises and acute stress decreases plasma zinc levels, plasma levels may not accurately reflect total body zinc in SS patients. Although the best measure of body zinc remains controversial

(Hambridge 1988) low zinc levels in erythrocytes (Prasad *et al*, 1975, 1976; Prasad and Cossack, 1984), hair (Karayalcin *et al*, 1974, 1979), and neutrophils (Warth *et al*, 1981; Prasad and Cossack, 1984) are consistent with a zinc deficiency in SS disease. The mechanism for this deficiency is unknown but dietary zinc intake of SS patients, based on a three day food record (Phebus *et al*, 1988; Enwonwu *et al*, 1990; Gray *et al*, 1992) or 28 dietary recall (Tangney *et al*, 1989), appears to be above recommended daily allowances and similar to that of control subjects. Intravascular haemolysis of erythrocytes rich in zinc and high urinary losses (Prasad *et al*, 1975, 1976; Niell *et al*, 1979; Enwonwu *et al*, 1990) (secondary to increased glomerular filtration and reduced tubular reabsorption) could therefore be the mechanism for zinc deficiency in SS disease.

The association of zinc deficiency with growth retardation in normal children (Carter *et al*, 1969; Golden and Golden, 1981a) prompted attempts to link zinc deficiency with growth and developmental delay in SS patients. Abbasi *et al* (1976) reported a significant correlation of plasma zinc with serum testosterone levels in 17 SS adult males and Phebus *et al* (1988) found lower serum zinc levels in 24 growth impaired (height for age less than the 5th centile for NCHS standards) compared to normally growing SS children. Neutrophil zinc levels correlated with body weight in 24 SS adolescents (Prasad and Cossack, 1984) and carbonic anhydrase activity (a zinc dependent enzyme) was significantly lower in 11 growth retarded compared to 12 normally growing children with SS disease (Daeschner *et al*, 1981). However, most studies in prepubertal children have failed to detect a relationship between plasma zinc levels and growth performance (Daeschner *et al*, 1981; Finan *et al*, 1988; Abshire *et al*, 1988) although slow healing of chronic leg ulceration (but beneficial effect of zinc supplementation, Serjeant *et al*, 1970), abnormal zinc dependent dark adaptation of vision (Warth *et al*, 1981), and abnormal leucocyte function (Ballester and Prasad, 1983; Carpentieri *et al*, 1983; Tapazoglou *et al*, 1985) all suggest a generalised effect of zinc deficiency in SS disease.

The growth promoting effects of zinc supplementation on growth retarded AA adolescents (Ronaghy *et al*, 1974) and children recovering from malnutrition (Golden and Golden, 1981b) has led to the initiation of zinc supplementation trials in SS disease. Zinc supplementation has been reported to increase the levels of testosterone and the testosterone response to GnRH (Prasad *et al*, 1981), increase the growth of body hair (Prasad *et al*, 1975), and to promote height and weight gain (Prasad and Cossack, 1984) in SS patients. However, the extremely small numbers of subjects (4 in the study of Prasad and Cossack, 1984), short supplementation periods and insufficient information concerning the growth patterns of patients and controls prior to supplementation greatly limit the usefulness of data obtained from these trials.

Folic acid and Iron

Since Zuelzer and Rutzky (1953) first described megaloblastic change in a child with SS disease, folic acid supplementation has become commonplace in the long term management of patients. High folate requirements for accelerated erythropoiesis are likely to be further increased during rapid growth and, theoretically, a folate deficiency could contribute to the growth retardation of SS children. Folic acid supplementation appeared to accelerate growth and the onset of puberty in 5 growth retarded SS adolescents who did not show megaloblastic change (Watson-Williams 1962) and low serum folate levels were noted in 6/8 growth retarded children with SS disease (Jimenez *et al*, 1966). However, Liu (1974) failed to find a relationship between serum or red cell folate levels and age adjusted weight and height. The only double blind, randomised control trial of folate supplementation also found no change in height or weight velocity of children (aged 6 months to 4 years) after 1 year of supplementation, arguing against a role for folate deficiency in the growth failure of Jamaican SS children (Rabb *et al*, 1983).

An adequate supply of iron is essential for normal growth and although iron deficiency is increasingly recognised in SS disease (Vichinsky *et al*, 1981), its role in growth retardation has not been investigated.

Other Vitamin Deficiencies

The levels of several vitamins have been reported to be low in SS disease, including vitamin E (Chiu and Lubin, 1979; Natta and Machlin, 1979; Tangney *et al*, 1989), pyridoxine (vitamin B6) (Natta and Reynolds, 1984), vitamin B12 (Osifo *et al*, 1983, 1984), riboflavin (Mankad *et al*, 1982), vitamin C (Jain and Williams, 1985), serum carotenoids (alpha, beta and cryptoxanthin) (Natta *et al*, 1988; Tangney *et al*, 1989) and vitamin A (Finan *et al*, 1988; Gray *et al*, 1992). The evidence for vitamin deficiencies in SS disease, however, is often inconclusive and since dietary intakes appear to be adequate, the mechanism for low serum levels unclear. Furthermore, with the exception of vitamin A, low vitamin levels have been investigated for their anti-oxidant properties and not as requirements for growth.

Finan *et al*, (1988) reported low levels of vitamin A, retinol binding protein, prealbumin, and zinc in children with sickle cell disease (37 SS, 2 SC and 1 with SB^0 thalassaemia) compared to 10 normal AA children, but the significance of this observation is uncertain. Although vitamin A is essential for normal growth, low serum levels may indicate a poor general nutritional state and not a specific growth limiting nutrient. In this study only serum retinol binding protein levels were lower in growth retarded children with sickle cell disease (n=20) compared to those matched for age and sex but growing normally. Therefore the authors concluded that it was not possible to explain poor growth in sickle cell disease solely on the basis of the nutritional factors studied.

Macronutrients and Growth

The pattern of growth in children with SS disease (low weight and height, reduced skinfold thicknesses, and a delay in skeletal maturation and pubertal development)

is consistent with poor nutrition during childhood (Eveleth 1985; Reed *et al,* 1987). Recent studies have therefore focused on the possible role of protein and/or calorie deficiency in impairing growth.

Energy Balance

The metabolic requirements of increased erythropoiesis and a hyperdynamic circulation as a consequence of anaemia might be expected to increase protein and energy demands in SS disease. An increase in metabolic activity is likely to increase basal energy expenditure and Odonkor *et al* (1982a) reported a 23% increase in the basal metabolic rate of 8 African male patients (aged 14-16 years) compared to 5 AA controls of a similar age. This increase could not be explained by a significant genotype difference in T3 and T4 levels and the authors postulated that increased pulmonary ventilation as a result of underlying hypoxia was the basis of their observation. However, the validity of these data is limited since patients and controls were matched by chronological age and not by their stage of sexual maturation which may affect basal or resting metabolic rate (RMR) (Cunningham 1980). In addition, basal metabolic rate was calculated on the consumption of oxygen only and the respiratory quotient (RQ) was assumed to be similar in SS and AA subjects. Although a 22% increase in RMR was also observed in 6 Jamaican adult male patients compared to AA controls of a similar age (Badaloo *et al,* 1989), the interpretation of both Jamaican and African studies is limited by small study size, ascertainment biases, lack of reference data for lean body mass, and lack of data on female subjects. Similar criticisms can be applied to a more recent study by Gray *et al* (1992), who reported a 23% increase in the RMR of SS children aged between 6-11 (mean 7.9) years (although this study did include 3 female subjects and attempted to correct RMR for the fat free mass calculated from skinfold measurements) and to the increase in RMR reported in North African adolescent girls with SS disease (Apatu *et al,* 1988).

Since RMR is the major determinant of daily energy expenditure (Garrow 1985), a high RMR could have a considerable impact on the energy requirements

and energy expenditure of patients with SS disease. Unless energy is conserved by reducing physical activity, or made available by increasing energy intake, an energy shortage could develop. No data are available on the physical activity of patients and data on dietary intake are also limited. Early anecdotal reports of anorexia in SS patients (Scott *et al*, 1955) were not confirmed by three recent studies that found no difference in the energy intake of SS and AA subjects. Phebus *et al* (1988) used a 3 day dietary record kept by the patient to show that the energy intake of 80 SS children was 99% of the recommended daily allowance and similar to 44 sibling controls. Enwonwu *et al* (1990), using a 3 day dietary record, also reported no statistically significant difference in the energy intakes of 22 SS adults and 13 AA controls, and finally Tangney *et al* (1989), using a 28 day questionnaire, found similar energy intake in 15 SS adults and 7 age and sex matched AA controls. In contrast, Gray *et al* (1992) reported a 28% increase in energy intake of nine 6-11 year old SS children compared to controls of a similar age, and in the only study to address both energy intake and expenditure, the ratio of energy intake to RMR in patients (1.43) was similar to that of AA controls (1.39).

Only one study has assessed the dietary intakes of growth retarded children with SS disease, Finan *et al* (1988) reporting similar energy intakes in growth retarded (height and weight below the 50th centile for NCHS reference children for the preceding 2 years) and normally growing SS children (height and weight between the 50th and 95th centiles). Interpretation of this study, however, is difficult since only half of the 20 children in each group completed the 3 day record of food intake.

Despite the importance of accurately assessing dietary intake in SS disease most studies can be criticised for failing to match patients and control subjects for socioeconomic status, and for using methods to estimate habitual food intake that have systematic errors of the same order of magnitude as likely genotype differences in nutrient intake.

Bingham (1987) estimated that the coefficient of variation of differences between estimated rather than actual weight of food may be 50% for foods and 20% for nutrients. Using variances from a British sample, she calculated that for a 3 day dietary record, a sample size of 100 subjects would be required to detect a 10-16% difference (significant at the 5% level) between the means of 2 groups. The dietary record is therefore unlikely to detect small deficiencies in energy intake between SS patients and AA controls, and although a 3 or 5 day weighed intake could detect any large genotype difference, this more accurate technique has not been used with SS patients. Further, even a weighed intake may fail to detect a small dietary deficiency which could have a cumulative effect and retard growth over several years. Measuring dietary intake in the steady-state of SS disease also ignores the contribution of anorexia induced by infections and bone pain to impaired energy balance in patients.

Nitrogen Metabolism

A high red cell turnover is likely to have significant impact on the metabolism of nitrogen in SS disease. The estimated increase in the rate of effective haemoglobin synthesis from 6.25 g/day to 40 g/day (Serjeant 1974) and corresponding increase in nitrogen requirements from 15 mg to 116 mg of nitrogen ($N.kg^{-1}.d^{-1}$) may also affect nitrogen balance in these patients.

Protein Turnover

Using a continuous infusion of C-14 lysine, Waterlow (1967) first demonstrated a higher protein turnover in SS disease (mean of 526 mg $N.kg^{-1}.d^{-1}$ in 2 adult male patients vs 260 mg $N.kg^{-1}.d^{-1}$ in a control subject). Although differences in race, age, and activity levels of subjects could have been contributing factors, the increase in protein turnover in SS disease was greater than the theoretical increase arising soley from the higher turnover of haemoglobin. However, after controlling for these factors under careful experimental conditions, Badaloo *et al* (1989) observed a similar increase in protein turnover (900 mg $N.kg^{-1}.d^{-1}$ in 6 adult males

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with SS disease vs 500 mg $N.kg^{-1}.day^{-1}$ in 6 AA controls) and postulated that metabolic requirements for cellular components of erythropoietic tissue other than haemoglobin could contribute to this genotype difference. A fall in protein turnover after splenectomy for hypersplenism (Badaloo *et al*, 1991; measured using N-15 glycine as a tracer and the same technique of Picou and Taylor-Roberts, 1969) was consistent with this hypothesis, since red cell turnover also falls following splenectomy (Emond *et al*, 1984). However, the small number of patients studied and the lack of a correlation between the change in protein turnover and the improvement in haematolgy suggests that this hypothesis requires further investigation.

Nitrogen Balance

Studies of nitrogen balance are consistent with differences in nitrogen metabolism in SS disease. Odonkor *et al* (1984) observed a negative nitrogen balance in 9 adolescent SS males even at the highest of three nitrogen intakes (90, 135, 180 mg $N.kg^{-1}.d^{-1}$). In contrast, 5 age and sex matched AA control subjects were in positive balance at the lowest of these intakes, suggesting a higher nitrogen requirement to maintain nitrogen balance in SS disease. Greater faecal nitrogen loss at all 3 levels of protein intake in patients suggested reduced absorption of dietary protein in SS disease although matching SS and AA adolescents by chronological age and not pubertal stage limited the validity of these comparisons. Patients were also fed fewer calories (total or per kg body weight, mean: 186.8 $kJ.kg^{-1}.d^{-1}$) than AA controls (211.6 $kJ.kg^{-1}.d^{-1}$), a discrepancy that probably arose because energy intake based on habitual energy intake (estimated from a dietary history) is likely to differ between subjects at different stages of maturation.

The source of increased faecal nitrogen in SS disease remains unclear. Jackson (1984) calculated that increased bilirubin production secondary to a high rate of haemolysis could explain only 10-15% of the increase in faecal nitrogen. Studies of urea metabolism (Jackson *et al*, 1988) also suggest that hydrolysis of

endogenously synthesised urea rather than malabsorption of dietary nitrogen may be the source of faecal nitrogen in SS disease.

Urea Kinetics

Using an intermittent infusion of Urea-N^{30}, Jackson *et al* (1988) demonstrated a marked difference in the handling of urea in SS disease. Adults with SS disease had increased urea production, both in absolute terms and relative to dietary intake of nitrogen. A smaller proportion of total urea production was excreted in the urine (40%) and there was a high fixed rate of hydrolysis of urea in the bowel. Approximately half of the nitrogen released by the hydrolysis of urea was resynthesised to urea and, assuming that all the increase in faecal nitrogen in SS disease was derived from urea, a further 10% may have been lost in the stool. The fate of the remaining nitrogen was not known and the authors suggested that this may have been available for protein synthesis.

The factors controlling the excretion and hydrolysis of urea in the bowel compared to the urine are poorly understood. The smaller proportion of dietary nitrogen excreted as urea nitrogen in the urine of patients (approximately 30% compared to 70% in normal subjects) was similar to that in AA subjects whose protein intake was insufficient to meet demand, suggesting that a relative protein deficiency could alter urea metabolism in SS disease. Alternatively, intestinal bacterial overgrowth could increase urea hydrolysis in the bowel and although there is no direct evidence of this condition, elevated fasting hydrogen gas levels in some SS patients were consistent with this hypothesis (Heyman *et al*, 1989).

The pathogenesis of increased urea production in SS disease is uncertain. Urea production was higher in patients even after accounting for the recycling of nitrogen released from the hydrolysis of urea in the bowel, arguing against inefficient absorption and digestion of dietary protein, but consistent with inefficient nitrogen metabolism increasing urea production. An imbalance between metabolic requirements and dietary supply of certain amino acids could decrease the efficiency of nitrogen metabolism and increase urea production and Jackson

(1984) has postulated that a relative deficiency of glycine may contribute to abnormal nitrogen metabolism in SS disease.

Specific Amino Acid Deficiencies

Haemoglobin is structurally an unbalanced protein, devoid of isoleucine, but rich in valine and leucine. Synthesis of the haem ring also requires 8 molecules of glycine which are lost to the body as bilirubin. Although as a non-essential amino acid glycine can be readily synthesised, requirements in SS disease may exceed the capacity of synthesis and dietary supply, leading to a glycine deficiency. Increased urinary excretion of 5-oxoproline (an index of glycine deficiency, Jackson *et al*, 1987) supports a marginal balance of glycine in SS disease. Glycine requirements are also high during periods of rapid growth (Persaud *et al*, 1987) and therefore competing demands of erythropoiesis and growth for glycine could contribute to the growth retardation of patients. Studies measuring 5-oxoproline in hypersplenic SS patients, who show marked erythropoietic expansion and catch up growth following splenectomy, could therefore help to clarify the role of glycine deficiency in SS disease.

Measurements of fasting plasma amino acid concentrations support specific amino acid deficiencies in SS disease (Enwonwu *et al*, 1990). Nineteen SS adults had significantly lower plasma concentrations of arginine (-38%), leucine (-32%), valine (-28%) and histidine (-32%) than 19 age matched AA controls on comparable nitrogen and energy intakes. Low plasma amino acid concentrations were not secondary to an exaggerated renal loss but probably a result of increased utilisation in tissues. A three to four-fold increase in 24 hour urinary orotate secretion also suggested that arginine may be particularly deficient although interpretation of these data are unclear since the validity of orotic acid as a good indicator of arginine deficiency in humans rather than animals has been questioned (Carey *et al*, 1987). A low plasma concentration of an amino acid is not definitive of a deficiency (Waterlow and Fern, 1981) and the 20% decrease in plasma concentration of isoleucine (which is absent in haemoglobin) suggests that

increased haemoglobin synthesis alone is unlikely to account for genotype differences in plasma amino acid concentrations.

Nutritional Supplementation and Growth

The most direct evidence of the influence of nutrition on growth retardation in SS disease is the small study by Heyman *et al* (1985) that investigated 5 SS boys (aged 3-16 years), who had habitual energy and protein intake above daily recommended allowances, and height and weight below the 5th percentile (according to NCHS centiles). For 2 patients nutritional intervention was confined to dietary counselling plus supplemental iron, zinc, folate and vitamin E; the third patient received oral supplemental formula providing 400 kilocalories (1.7 MJ) daily at bedtime, patient 4 had daily nasogastric supplements of 143 kilocalories/kg (0.6 MJ) energy and 2.7g protein/kg for six weeks, and patient 5 1060 kilocalories (4.4 MJ) energy and 36.7g protein via nocturnal nasogastric feeding for 11 months. At the end of the intervention period, nasogastrically supplemented boys had a marked increase in height velocity and reduction in pain crises and infections, suggesting a correction of a nutritional deficiency. Oral supplements had no effect on growth (probably because compensatory anorexia between supplements prevented a substantial increase in energy intake) but improved the clinical course, and mineral and vitamin supplements had no effect on growth or clinical course. The authors postulated that an energy rather than a specific nutrient deficiency was likely to retard growth in SS children but the small numbers of patients studied and the study design make it impossible to determine which factors were responsible. Furthermore, as noted by Luzzatto (1985), this study was deficient in that there was no information on iron or folate status of patients, energy intakes of non-supplemented children, or on other factors such as the size of the spleen, HbF levels and alpha thalassaemia status all of which influence growth in SS disease.

:. Type II deliveries ++

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Summary

A shortage of one or more nutrients could lead to poor growth in SS children. Although, such a deficiency could arise by a reduced nutrient intake or impaired intestinal absorption there is little evidence for the latter and most available data suggest that dietary intake in SS patients is above daily recommended allowances and similar to that of AA controls. Nonetheless, it is becoming increasing apparent that a nutrient intake that is sufficient for a haematologically normal child may be inadequate for a child with SS disease.

? meaning

Several studies suggest increased metabolic demand in SS disease. Resting metabolic rate is increased by 20% and protein turnover is almost doubled. Nitrogen balance may be difficult to maintain at high nitrogen intakes and the metabolism of urea is altered, some changes being similar to normal patients whose protein intake is insufficient to meet demand. The factors responsible for these changes are unclear, but the fall in protein turnover after splenectomy for hypersplenism suggest that a high red cell turnover may contribute to these metabolic differences. A high rate of erythropoiesis in SS disease may therefore increase requirements for protein, energy or specific amino acids such as glycine.

The only supplementation trial (Heyman *et al*, 1985), although showing impressive improvements in growth, failed to show which nutrients were growth limiting. However, this study suggested a need to investigate further the possible role of nutritional deficiency in the growth retardation of children with SS disease.

Garden
Type II .

CHAPTER 2: DETERMINANTS OF GROWTH;

ANALYSIS OF DATA FROM THE JAMAICAN COHORT STUDY

What did researcher do?

METHODS

Prior to the advent of community based studies the traditional clinical picture of SS disease was of a severe chronic illness with a poor prognosis. Reports of asymptomatic cases and long term survival in adults (Serjeant 1974) however conflicted with the traditional understanding of SS disease and led to recognition of a wide variation in disease severity. It was soon apparent that early epidemiological studies were probably biased since severely affected SS patients were more likely to present to clinics and be selected for study than those with relatively benign disease. The need to document the natural history of SS disease in a more representative sample of patients therefore lead to the Jamaican cohort study.

The Cohort Study

Suitability of Jamaica

A high birth rate and 10% frequency of the sickle cell gene in population of 2.3 million, predominantly (95%) of West African origin, made Jamaica well suited to a natural history study of SS disease. An incidence of clinically significant sickle cell disease as high as 1 in 150 births, including 1 in 300 with SS disease, 1 in 500 with SC disease, 1 in 3000 with sickle-beta$^+$ thalassaemia, and 1 in 7000 with sickle-beta^0 thalassaemia (Serjeant 1985) provided a varied study population. The high proportion of births (13,819 births out of 61,000 [23%] on the Island in 1974) concentrated at the main government maternity hospital in Kingston (Victoria Jubilee Hospital) allowed neonatal detection of a large cohort of patients and the relative lack of primary care facilities in Jamaica encouraged regular patient attendance at the Medical Research Council Sickle Cell Clinic, increasing contact and rapport between patients and staff. Finally, good communications throughout Jamaica permitted contact with patients who had moved from the Corporate Area.

Aim

The aim of the Jamaican study was to document the natural history of SS disease and to investigate the clinical and haematological determinants of disease severity. Patients were detected by cord blood screening and followed at the sickle cell clinic.

Cord Blood Screening

Screening of 100,000 consecutive live births (representing 95% of all normal deliveries) at the main government maternity hospital between June 1973 and December 1981 detected all patients with sickle cell disease. At the time of every delivery, 2-10 ml of cord blood was collected or a heel prick sample taken if any infant was inadvertently omitted. The first 125 children diagnosed as SS disease were each matched with two AA controls of the same sex, born close in time to the index case yielding 250 control subjects. A total of 315 children with SS disease, 201 with SC disease, 13 with sickle cell-beta^o thalassaemia, 34 with sickle cell-beta$^+$ thalassaemia, 20 with other sickle cell syndromes and 250 with a AA genotype were thus recruited.

Diagnostic Techniques

Since beta chain synthesis accounts for only a small percentage of total haemoglobin chain synthesis at birth (Serjeant *et al*, 1974), techniques used for cord blood screening had to be capable of detecting small amounts of beta chain variants such as HbS and HbC. The need to detect HbA to distinguish sickle cell trait from SS disease, however, precluded the use of sickling and solubility tests for HbS or the use of traditional methods of haemoglobin electrophoresis which could readily demonstrate HbS but not separate HbA from HbF. Agar gel electrophoresis, although capable of separating HbA from HbF, was also unsuitable since it was time consuming and did not allow recognition of rarer haemoglobin variants that had similar mobility to HbA and HbS. Therefore a technique which separated haemoglobin A, F and S on cellulose acetate using an alkali buffer was used to initially screen all cord blood samples followed by further

investigation of all abnormal haemoglobin bands using agar-gel electrophoresis (Serjeant *et al*, 1974). Close agreement of the observed frequency of haemoglobin genotypes with values predicted from the gene frequency in the population confirmed the accuracy of this method although diagnosis of patients with sickle cell-beta^{o} thalassaemia or hereditary persistence of fetal haemoglobin required electrophoretic studies in parents or observations of the evolution of haematology and HbA_2 over the first year of life.

Clinical Data Collection

All cohort children were initially seen at the sickle cell clinic at three monthly intervals coinciding with their birthdays. (The schedule was changed to 6 monthly visits in AA subjects at age 5 years to reduce interference with schooling). At each visit clinic staff measured the patient's height (see growth study), took a clinical history and performed a physical examination. Particular attention was paid to the child's health since the previous visit and whether the child had been seen by another doctor or admitted to hospital, to the presence of pallor, jaundice, cardiomegaly, a heart murmur, hepatomegaly and splenomegaly (measured along the long axis from the splenic tip to the left costal margin), as well as to specific presenting symptoms. At each birthday the patient's recent address and socioeconomic status, based on a score previously used in Jamaica (Alleyne *et al*, 1979), was recorded. This score recorded the source of household water, the type of sanitation, and the number and type of electrical appliances in each household, but ignored paternal income since this is frequently an unreliable index in single parent families in Jamaica.

Mothers were encouraged to attend the sickle cell clinic routinely and whenever medical attention was required and a comprehensive paediatric service was provided with the aid of specialist paediatric clinics and inpatient facilities at the University hospital. Essential medication was given without charge and a full immunisation programme was available. Every opportunity was taken for education of patients and their parents, particularly with regard to the genetic

implications of carrying the S gene, family planning, prevention of cold precipitated painful crises, importance of good nutrition, and the need for early medical attention when sick. Parents were also taught to feel for the spleen, which encouraged earlier diagnosis of acute splenic sequestration and reduced the usually high mortality from this condition, and younger children were given a monthly injection of long acting penicillin to reduce the risk of pneumococcal sepsis.

Cohort patients defaulting from the study were contacted by telegram, letter, or a home visit by a member of the clinic staff, and encouraged to attend by providing bus fares when required. In addition two clinics in the west of the Island, visited once a month by Unit staff, encouraged attendance by patients who had moved from Kingston. A careful record was kept of all patients lost to the cohort study due to death or emigration (34 or 11% of cohort by 1992).

Assessment of Morbidity

Morbidity was assessed as the number of specific clinical events (hospital admissions, sick visits, painful crises, and episodes of acute chest syndrome or of acute splenic sequestration). Although the first of these indices may be influenced by bed availability and the second by parental anxiety, the combination gave a better approximate indication of overall severity than a single index. As patients in the cohort study relied almost exclusively on the sickle cell unit for their medical care from birth, the record of morbidity was likely to be reasonably accurate and complete.

Morbidity data were collected by recording all clinical events at each clinic visit and asking patients to recall events occurring between visits. Painful crisis was defined as bone pain severe enough to limit activity, acute chest syndrome as a history of cough and fever with clinical and/or radiological evidence of pulmonary consolidation and acute splenic sequestration as an acutely enlarging spleen associated with a fall in haemoglobin from steady-state levels of at least 20 g/L, and evidence of increased bone marrow activity (Topley *et al,* 1981). For the

purpose of analysis two or more episodes of a clinical event occurring within 1 week of each other were regarded as a single event.

Haematological Techniques

Venepuncture samples were taken at six month intervals in patients with sickle cell disease, annually in AA control subjects, and when clinically indicated in all children. Haematologic indices, including total Hb, red blood cell count (RBC), mean cell volume (MCV), mean cell haemoglobin (MCH), haematocrit, mean cellular haemoglobin concentration (MCHC), platelet count and total nucleated cell count, were measured on a Coulter model S plus 4 (Coulter Electronics, Hialeah, Florida). The total nucleated count was corrected for nucleated red blood cells to give the white cell count. Reticulocyte counts were determined by standard microscopic techniques after incubating whole blood with brilliant cresyl blue for 30 minutes at $37^{O}C$ and were expressed as percentage reticulocytes of 500 RBC. Haematological indices taken at the time of clinically well visits, and excluding any values for 3 months after a blood transfusion were taken as 'steady-state' values and used for analyses.

At each birthday visit, a sample of serum was stored at $-70^{O}C$, fetal haemoglobin concentrations measured using the alkali denaturation method of Betke *et al* (1959) and serum iron and iron binding capacity measured using the method of Beale *et al* (1962). Special investigations included the number of F-cells, and F-reticulocytes, measured by the method of Dover *et al* (1978a) in all cohort children presenting to the clinic over a 3 month period, and the number of alpha haemoglobin genes which were available in 232 cohort children (representing all those available in Jamaica after 1976).

Data Handling and Computing

All clinical and haematological data were stored on a Unisys A4 main frame computer. Doctors inserted clinical data via computer terminals and haematological data were transmitted directly from the Coulter S plus 4. The

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database was continually updated with demographic, clinical (including growth data) ophthalmological and haematological information.

The Growth Study

Aim

Data from the cohort study provided an ideal opportunity to study the growth of a representative sample of children with SS disease but previous analyses in the cohort study were confined to genotype comparisons of growth, body shape and skeletal maturation in children aged 6 years or younger. As all children in the cohort study had passed the age of 10 years, the aims of the present study were to assess the pattern of adolescent growth, to investigate the determinants of growth, and to clarify the role of abnormal haematology in the growth of SS children.

Who did this?

Anthropometric Measurements

Heights and weights of all cohort children were measured at 3 month intervals arranged to coincide with birthdays and on any sick visits to the clinic. Sitting height, arm span, arm circumference, and triceps, biceps, subscapular and suprailiac skinfold thicknesses were measured at six month intervals from the age of 8 years in the first 125 SS children and their AA controls. Pubertal development was also assessed at these 6 month visits by Tanner staging and testicular volume measured using a Prader orchidometer. Anthropometric measurements were expanded on the eighth birthday of all children to include chest circumference, anterior-posterior and lateral chest diameters, inter-acromial and inter-cristal diameters, and an assessment of bone age using the Tanner-Whitehouse 20 bone method.

Measurement Techniques

Height and weight measurements were made using standard protocols (Cameron 1984) by nursing or medical staff trained in the techniques involved and all other anthropometric measurements by medical staff. Inter-observer measurement variation was a potential source of error but as it was not feasible for all

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measurements over 18 years to be done by a single observer, great emphasis was placed on standardisation of technique. All measurements were performed in triplicate and reported as the mean value. Intra-individual measurement variation (for observer Dr A Singhal) was assessed by a test-retest study in which 2 sets of anthropometric measurements were made in a blind manner, 6 hours apart, in 22 consecutively attending children (Table 1). Values for comparison of intra-observer relaibilities form other studies are also given in Table 1.

Kres. Prev. publ. data?

Measurement	Mean difference (d)		Technical error of measurement $[Ed^2/2n]^1$		Coefficient of variation $CV= Ed^2/2n/X^1$	
Height (cm)	0.25	$[2.0]^2$	0.23	$(0.494)^3$	0.156	(0.302)
Weight (kg)	0.15		0.15	(1.173)	0.421	(2.119)
Arm span (cm)	0.42	[0.5]	0.43	[0.30-0.60]	0.282	
Arm circumference (cm)	0.19		0.18	[0.10-0.40]	0.891	(1.358)
Sitting height (cm)	0.48	[0.5]	0.45	[0.10-0.30]	0.505	(0.631)
<u>Skinfolds (mm)</u>						
Triceps	0.59		0.63	[0.80]	7.802	(6.51)
Biceps	0.25		0.25	[0.20-0.60]	5.795	
Subscapular	0.35		0.44	[0.80-1.53]	6.440	(18.30)

Table 1

Intra-observer Reliabilities for Anthropometric Measurements in 22 SS Children

E = sum of; d = difference between two measurements, n = number of paired measurements; X= overall mean of measurements
[1] methods for calculation Cameron, 1984
[2] values for comparison of measurement reliabilities in square brackets from Lohman 1988
[3] values for comparison of measurement reliabilities in round brackets from the NCHS survey in Cameron, 1984

Height – was measured as supine length using a supine stadiometer accurate to 1 mm, until the children were old enough to co-operate with measurement of standing height. Standing height was measured on a regularly calibrated Harpenden wall mounted stadiometer (Holtain Ltd, Crymmych, UK), accurate to 1 mm.

Sitting height – was determined using a sitting height table (Holtain Ltd, Crymmych, England) with the subject's head in the Frankfurt plane, the back straight and forming a right angle with the horizontal upper surface of the thighs, and the feet supported so that the tendons of the biceps femoris just cleared the table. Subischial length was derived from standing height minus sitting height.

Weight – was measured in infants using a set of baby balance scales (Detecto Inc., Brooklyn, New York) accurate to one ounce (0.03 kg). Weights were measured in pounds and ounces and subsequently converted to kilograms. Weights in older children were measured on a lever balance (Detecto Inc., New York) accurate to 0.1 kg.

Arm span – was measured with the child standing straight and holding his arms as horizontal as possible.

Mid upper arm circumference – was determined by first identifying the mid-point between the olecranon and acromion with the left elbow bent at 90 degrees. The circumference was measured at this point with the arm extended, using a steel tape measure held firmly against the arm without indenting the skin.

Skinfold thicknesses – were measured using Holtain calipers (Holtain Instruments Ltd., Crymmych, UK) held in the right hand while raising a skinfold between the index finger and thumb of the left hand. Maintaining the pinch throughout, the

calipers were applied just below the pinch point, the grip on the handles relaxed and readings taken as soon as the dial had settled (after approximately 3 seconds). Readings were taken in triplicate and the mean recorded to the nearest 0.1 mm.. Triceps skinfold was taken at the mid point of the left upper arm over the posterior surface of the triceps in the vertical plane and subscapular skinfold was measured just below the angle of the left scapula with the skinfold aligned in the direction of the natural cleavage line of the skin. Supra-iliac skinfold was measured just above the anterior iliac crest in a horizontal or oblique plane along the natural line of the skin.

The age at menarche – was ascertained by prospective enquiry at each clinic visit. The exact date of menarche could usually be recalled but if only the month was remembered the 15th day of that month was used for analysis.

Editing of Growth Data

Growth data were edited (for over 27,000 observations) by plotting height and weight against age for all children and verifying outlying points. The commonest errors were clerical but no explanation could be found for 173 (0.6%) of observations which were therefore excluded.

Ethical Considerations

All studies were approved by the ethical committee of the University of the West Indies and all patients and their guardians gave informed and written consent for individual investigations. All procedures were non-invasive and venepunctures in addition to those required in the protocol were confined to those required for clinical purposes in SS patients. Children were free to leave any study but continued to receive medical care at the sickle cell clinic.

Summary

The neonatal screening of 100,000 deliveries provided a representative study group of SS children free from the ascertainment bias of symptomatic selected, clinic based populations. Selection of AA controls from the same population as SS subjects minimised socioeconomic differences between genotypes, and both SS and AA children were representative of the socioeconomic status of the urban population of Jamaica (Knight *et al*, personal communication). Longitudinal measurement of growth and haematological changes using standardised methods of data collection allowed assessment of the pattern and determinants of growth in SS disease.

Good care in this study. ..? representative

ADOLESCENT GROWTH AND PUBERTAL DEVELOPMENT

Adolescent growth is delayed in children with SS disease (Platt *et al*, 1984), but a normal adult height (Ashcroft *et al*, 1972) suggests that retarded skeletal maturation (Serjeant and Ashcroft, 1973) allows catch up in final height. The overall pattern of adolescent growth in SS disease is unclear since previous cross-sectional data (Ashcroft *et al*, 1972; Platt *et al*, 1984), fail to account for individual variation in the timing of the adolescent growth spurt. Simply taking means of individuals heights at different ages produces a mean growth curve which is lengthened and reduced in intensity but a more accurate quantitation of the growth defect in SS adolescents has been limited by the lack of longitudinal observations. The aim of the present study was to fit longitudinal height data from the Jamaican Cohort Study to a mathematical model (Preece and Baines model 1) (Preece and Baines, 1978) inorder to assess the effect of sickle cell disease on the pattern of adolescent growth and pubertal development.

Subjects and Methods

The study was confined to post-pubertal cohort children with height observations available to the age of 16 years. At the study date 76/315 SS children should have been eligible from their dates of birth but 21 had died, 5 had emigrated, and 3 were excluded because of a chronic disease other than sickle cell disease which might have affected growth. Three boys with extreme retardation of sexual maturation but growing normally for their bone age were also excluded since they were still prepubertal (Tanner stage 1) and their data could not be analysed using the Preece-Baines model 1. There remained 44 SS patients (mean age, SD: 17.9, 0.6 years; 21 males, 23 females) who were matched by age and sex to the closest 44 cohort children with SC disease (17.3, 0.8) and to 44 control AA children (17.9, 0.5).

Growth Data

Height was measured at 3 month intervals in SS and SC children and at 6 month intervals in AA controls (aligned with the subject's birthday) as previously described. Sitting height and subischial length were ascertained for AA and SS subjects as previously described and pubertal stage assessed by Tanner staging at six monthly clinic visits from the age of 8 years. The first sign of puberty (whether pubic hair or genitalia) was used for analysis since this is likely to be the most accurately recorded. To minimise any bias that may arise from SS children attending more regularly than AA controls, the age at Tanner stage 2 was calculated as the midpoint of the date at which pubertal changes (pubic hair or genitalia) were first observed and the last date at which children were prepubertal. The length of puberty was defined as the interval between first assessment as Tanner stage 2 and first assessment as Tanner stage 4 or 5 (pubic hair or genitalia).

Curve-fitting

Height data at 6 month intervals (± 8 weeks) from the age of 1 year were fitted by computer (using the least squares regression method) to the Preece-Baines Model 1 as described below:

$$h(t) = h_1 - \frac{2 (h_1 - h_q)}{\exp [S_0 (t - q)] + \exp [S_1 (t - q)]}$$

where h is height (cm) at age t, h_1 is final adult height, S_0 and S_1 are rate constants, q is a time constant and h_q is height at t = q.

This model has been extensively used to fit longitudinal data on height (Hauspie *et al*, 1980a and b; Mirwald *et al*, 1981) and has the advantage over other mathematical models of not requiring the subject's final adult height. The model resolves complex growth curves into a limited number of biologically meaningful parameters defining the adolescent growth curve of each individual and allowing comparison of the timing and degree of the adolescent growth spurt

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between individuals. The principle indices were the age at onset of the adolescent growth spurt (take-off, TO) and the age at peak height velocity (PHV). Other factors derived from the model included 4 indices of growth (age, height, height velocity, and percentage of adult height) at take off and at peak height velocity, predicted adult height, height gained between TO and PHV, and between PHV and adult height, and change in height velocity between TO and PHV.

no bone age needed?

Statistical Analysis

Genotype and sex differences in growth parameters were statistically assessed using the multivariate approach to Repeated Measures Analysis of Variance. Type 1 error was fixed at 5% for each omnibus null hypothesis and the significance level of each pairwise comparison was adjusted using Bonferroni's method. The mean values of the mathematical parameters describing individual curves were used to produce a mean constant curve for each genotype and sex group. For each individual, all the differences between observed and fitted points (residuals) were used to calculate a standard deviation of residuals. A small standard deviation indicates a better fit of the model for the individuals data.

Results

Curve-fitting

The model fitted the growth data of AA, SS, and SC subjects equally well and the mean of the standard deviation of the residuals did not differ significantly between genotypes (Table 2). The pooled residual mean of squares was 0.9 cm^2 for the whole data set and was similar for each genotype (0.9 cm^2 for AA, 1.0 cm^2 for SS, and 0.8 cm^2 for SC subjects).

Table 2							
Residuals Data for Fitting Preece-Baines Model I to Height							
Figures are mean (SD)							
		AA		SS		SC	
Number of	M	24.5	(4.2)	25.8	(5.3)	21.3	(5.6)
Data Points	F	24.1	(3.8)	25.3	(5.8)	22.3	(6.3)
Standard Deviation	M	0.75	(0.18)	0.79	(0.19)	0.80	(0.29)
of Residuals (cm)	F	0.87	(0.26)	0.94	(0.32)	0.70	(0.25)
SC patients had significantly fewer (p<0.05) data points than AA and SS subjects							

The Pattern of Adolescent Growth

The indices of adolescent height growth in the 3 genotypes are summarised in Table 3. Compared to AA controls, SS patients showed a delay in mean age at take off of 1.4 years (95% confidence intervals, CI: 0.8 to 2.0), a delay in mean age at peak height velocity of 1.6 years (95% CI: 0.9 to 2.3), and a lower height velocity at take off of 0.7 cm per year (95% CI: 0.3 to 1.0). Comparable figures in SC patients compared to AA controls were 0.3 years (-0.2 to 0.8), 0.4 years (-0.2 to 1.0), 0.3 cm/year (0.0 to 0.6), none of which were significant. No other significant genotype differences occurred. The mean predicted height and height velocity against age for males and females using fitted data (mean constant curve) are shown in Figures 1 and 2.

There were significant sex differences in height indices in all genotypes, males showing a later age at take off and at peak height velocity, greater height at TO and at PHV, a lower height velocity at TO, a greater height gain from TO to PHV, and a greater predicted adult height.

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Table 3

Table 3
Adolescent Height Growth in Sickle Cell Disease
Repeated Measures Analysis of Variance; Figures are mean (SD)

	AA CONTROLS		SS DISEASE		SC DISEASE		Effect tested	
	Boys	Girls	Boys	Girls	Boys	Girls	Genotype	Sex
1. Age at take off (TO) (years)	10.4 (1.1)	8.5 (1.1)	11.7 (1.1)	10.1 (1.0)	10.7 (1.2)	8.8 (1.0)	*	+
2. Height at TO (cm)	136.4 (7.0)	129.7 (6.5)	139.8 (8.0)	130.5 (7.8)	135.7 (6.1)	128.5 (7.7)		+
3. Height velocity at TO (cm/year)	4.4 (0.6)	5.1 (0.7)	3.9 (0.8)	4.3 (0.6)	4.2 (0.7)	4.8 (0.6)	*	+
4. % Adult height at TO	78.1 (2.4)	78.2 (3.3)	80.3 (5.6)	79.3 (3.2)	77.8 (3.7)	79.3 (2.1)		
5. Age at peak height velocity (PHV) (years)	14.2 (1.4)	11.6 (1.1)	15.5 (1.5)	13.5 (1.3)	14.5 (1.3)	12.1 (1.2)	*	+
6. Height at PHV (cm)	157.7 (6.1)	149.3 (5.2)	158.9 (13.4)	148.8 (7.2)	158.1 (7.4)	147.2 (7.8)		+
7. Peak height velocity (cm/year)	7.8 (1.6)	7.8 (1.3)	6.7 (2.0)	6.9 (1.3)	8.7 (1.8)	7.3 (1.1)		++
8. % Adult height at PHV	90.3 (1.5)	90.1 (1.9)	90.9 (2.6)	90.5 (2.2)	90.5 (1.8)	90.7 (1.3)		
9. Predicted adult height (cm)	174.6 (6.9)	165.8 (4.7)	175.2 (18.4)	164.5 (7.3)	174.7 (9.7)	162.2 (8.6)		+
10. Height gained TO-PHV (cm)	21.3 (3.1)	19.6 (3.6)	19.2 (8.1)	18.3 (4.4)	22.3 (4.6)	18.6 (2.2)		+
11. Height gained PHV to adult height (cm)	16.9 (2.8)	16.5 (3.2)	16.3 (6.5)	15.7 (3.7)	16.6 (3.7)	15.0 (2.4)		
12. Change in height velocity TO-PHV (cm/year)	3.4 (1.7)	2.7 (1.6)	2.8 (2.2)	2.7 (1.5)	4.5 (2.0)	2.5 (1.1)		++

* Overall genotype difference p<0.001. Paired comparisons showed differences were between AA v SS and SC v SS.
+ p<0.05; ++ within SC genotype only p<0.05

Figure 1a and 1b:
The mean height and height velocity for males derived from the mean of individual data fitted to the Preece-Baines model
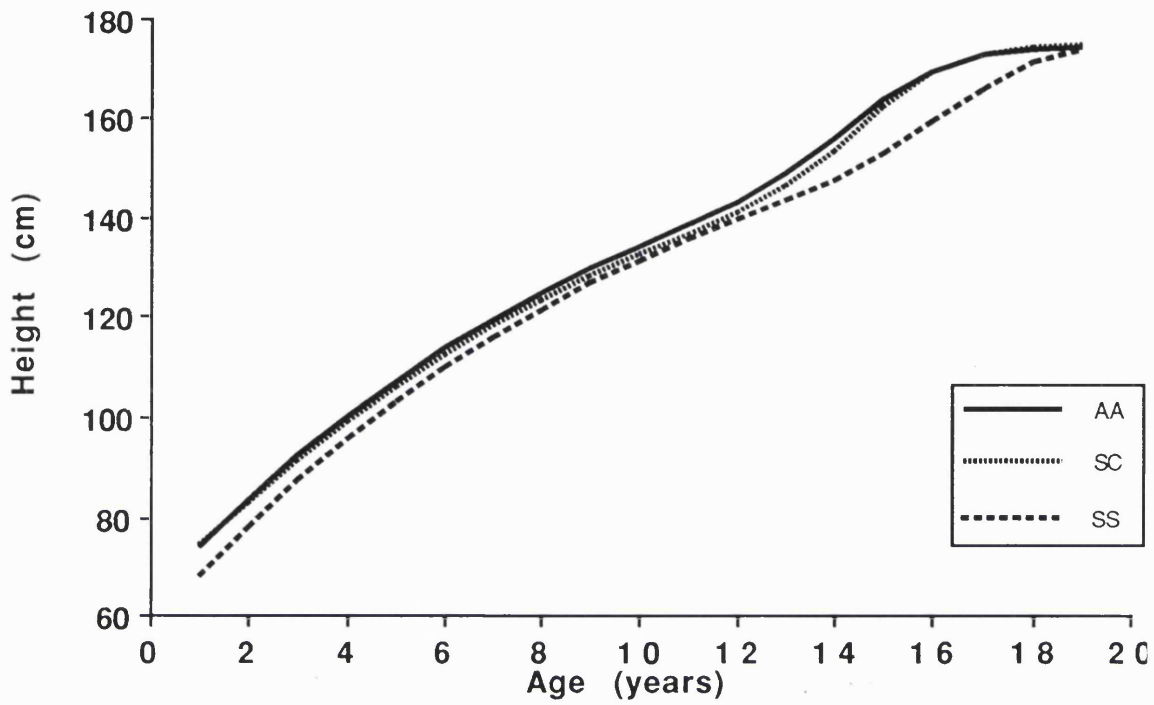
Figure 1b
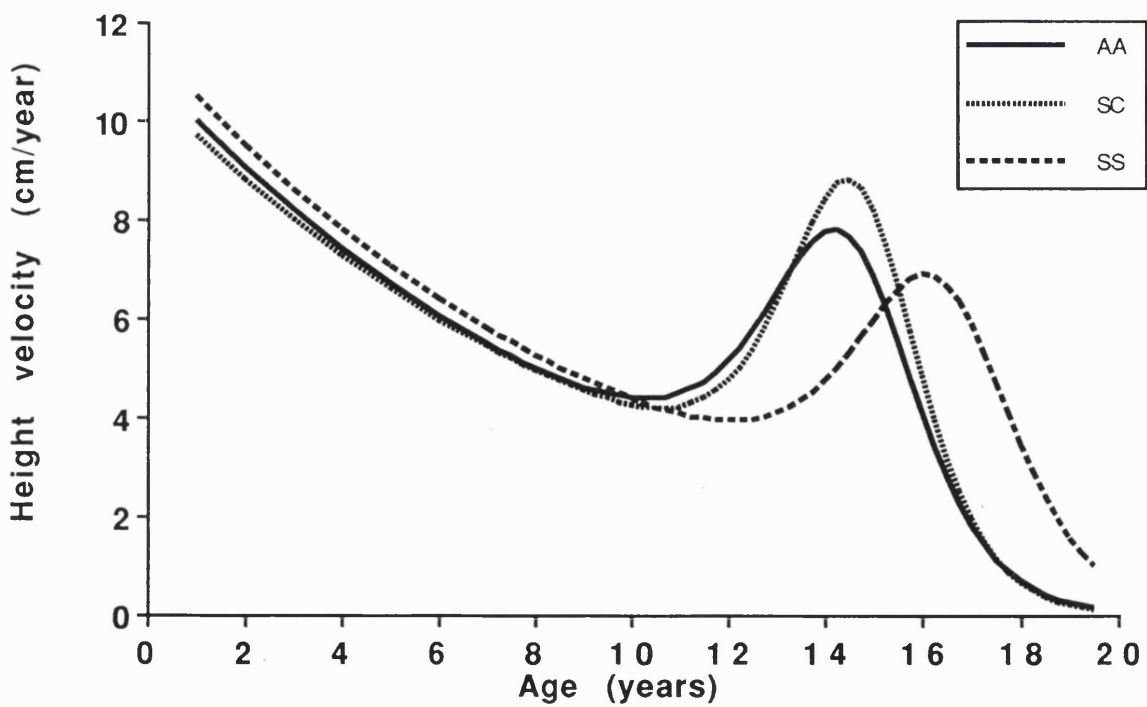
Figure 2a and 2b:
The mean height and height velocity for females derived from the mean of individual data fitted to the Preece-Baines model
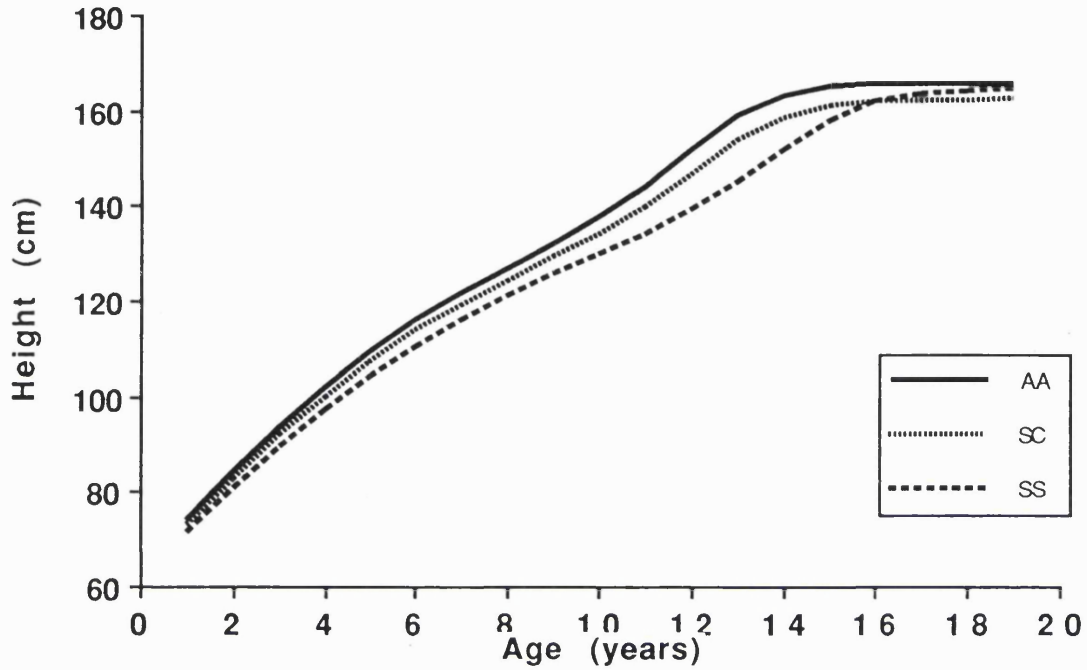
Figure 2b
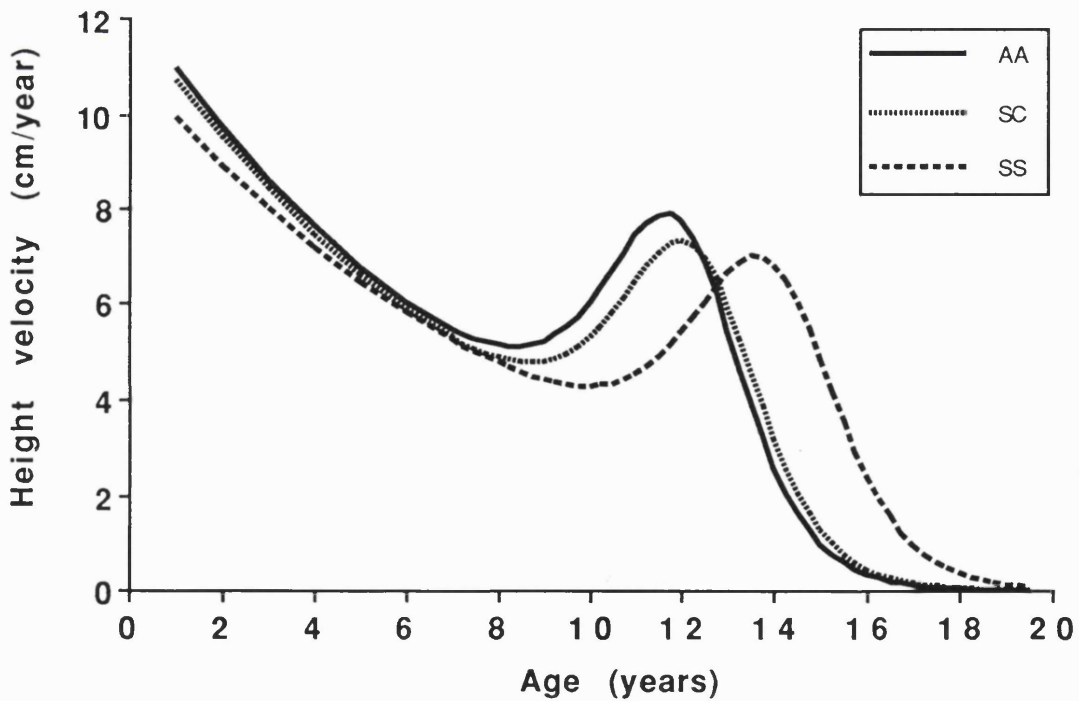
The sitting-height to subischial length ratio (available in 29 and 27 SS/AA pairs at age 16 and 8 years respectively) was significantly lower in SS subjects (mean, SD: 0.95, 0.053) than AA controls (mean, SD: 0.99, 0.065; p=0.010, n=29) at age 16 years, but not at age 8 years (mean, SD: 1.06, 0.054 for SS subjects vs 1.04, 0.076 for AA controls; p=0.29). There were no sex differences in this ratio.

At the last clinic visit 41 SS, 42 SC and 43 AA subjects had a height difference of <1 cm/year over the preceding year. Three SS and 2 SC patients were still growing faster than 1 cm/year and one AA subject had died, leaving 39 matched SS, SC, and AA subjects with similar minimal rates of growth. In this group there was no significant genotype difference in height for males (mean, SD: SS: 174.3, 7.4 cm; SC: 174.0, 6.8; and AA: 173.0, 7.9) or females (mean, SD: SS: 164.0, 6.8 cm; SC: 163.2, 7.4; and AA: 165.7, 4.9; Repeated Measures Analysis of Variance).

The Pattern of Pubertal Development.

The first pubertal changes (Tanner stage 2) appeared later in SS disease (mean age, SD: males: 12.8, 1.6 years; females: 12.0, 1.8 years) than in AA controls (mean age, SD: males: 11.1, 1.2 years; females: 10.1, 1.2 years) for the 42 pairs with available data (Repeated Measures Analysis of Variance p<0.001) but adjusting for the age at TO or PHV reduced the mean difference from 1.8 to 1.2 years (95% CI adjusting for TO; 0.5 to 2.0; 95% CI for PHV: 0.4 to 1.9). The delay in onset of puberty in SS patients compared to their AA controls correlated with the genotype delay in age at PHV (r=0.36, p=0.02) but the correlation with genotype delay in TO just failed to reach significance (r=0.30, p=0.054). In both genotypes, boys started puberty later later than girls (p=0.01). The length of puberty, defined as the time interval between the first appearance of Tanner stage 2 and Tanner stage 4 or 5, was also greater in SS subjects (mean, SD: 2.9, 0.4 years) compared to AA controls (1.9, 0.4 years; p=0.002, using Wicoxon's one sample test). There were no Tanner stage data for children with SC disease.

The age at menarche in girls with SS disease (mean, SD: 15.4, 1.3 years) was significantly later than girls with SC disease (mean, SD: 13.7, 1.7 years) and AA controls (mean, SD: 13.1, 1.3 years; p<0.001 for the overall genotype comparison). However after adjusting for the delay in the adolescent growth spurt (by using age at TO or PHV as a covariate in Repeated Measures Analysis of Variance) the genotype difference in age at menarche was no longer significant (the 2.3 year difference between SS and AA girls was reduced to 1.0 years [95% CI: -0.1 to 2.0] after adjusting for age at TO and to 0.6 years [95% CI: -0.4 to 1.6] after adjusting for age at PHV). The genotype differences in age at menarche between SS and AA, SS and SC, and AA and SC pairs were highly correlated with the genotype differences in age at TO or PHV (correlations ranged from 0.72 to 0.87, p<0.001 for all 6 comparisons).

Discussion

Interpretation of growth data from any population depends on the sample selected for investigation and the study design. Subjects from the cohort study were diagnosed at birth and should be a representative sample of children with sickle cell disease although observations in adolescence may be biased by early deaths of severely affected cases. Analysis of longitudinally collected height data using the Preece–Baines model 1 allowed assessment of individual variation in timing of the adolescent growth spurt and hence comparison of adolescent growth in the three genotypes. The quality of curve fitting in the present study was not as good as in previous applications of the model, the pooled residual mean squares of 0.9 cm^2 contrasting with figures of 0.19 cm^2 in the study by Preece and Baines (1978), and 0.46 (Hauspie *et al*, 1980a), 0.40 (Hauspie *et al*, 1980b) and 0.35 (Mirwald *et al*, 1981) in subsequent studies. A factor contributing to this variability may have been the analysis of data from as early as 1 year of age (since growth delay in SS disease is apparent as early as age 6 months) although better fits to the model may be obtained by restricting to data at later ages (Hauspie *et al*, 1980b). Other

factors may include the greater intrinsic inaccuracy in measuring young children, measurement error due to observer variation over the study span of 18 years, and technical variation arising from changing hairstyles.

The present data confirm delay in the adolescent growth spurt of SS children. The true delay in males may have been underestimated since 3 SS boys were excluded as their growth spurt had not started by age 16 years and therefore their pattern of adolescent growth could not be assessed. Adolescent growth in SS disease was otherwise normal and since height velocity falls prior to the adolescent growth spurt, the lower height velocity at TO probably reflects the delay in TO. Furthermore, the lack of significant genotype differences in attained height by age 17 years is in agreement with cross-sectional data suggesting normal height in Jamaican SS adults (Ashcroft *et al*, 1972) and consistent with delayed epiphyseal fusion (Serjeant and Ashcroft, 1973) allowing a catch up in height.

The more normal growth of SC compared to SS children has been reported (Platt *et al*, 1984; Stevens *et al*, 1986) and is confirmed by the height velocity growth curves. The later adolescent growth spurt of boys compared to girls of all genotypes was consistent with their later physical and sexual maturation but the pattern of adolescent growth and the degree of growth delay was similar in both sexes with SS disease.

The delay in onset of menarche in SS disease was appropriate for the overall retardation in adolescent growth, but the delay in appearance of the first pubertal changes persisted even after controlling for the later adolescent growth spurt. The reduced spinal (sitting) height to subischial length in SS children at age 16 years was characteristic of SS and normal children with delayed adolescent growth (Stanhope *et al*, 1992).

A normal but delayed pattern of adolescent growth in most children suggests that SS disease retards the onset of adolescent growth but not final height. Growth retardation from as early as six months of age but normal height gain during adolescence is consistent with factors affecting prepubertal growth

determining the timing of adolescent growth spurt and this hypothesis was assessed by investigating the determinants of delayed adolescent growth in SS disease.

Preece-Baines curves were fitted to a further 87 SS (44 males, 43 females) and 141 AA (64 males, 77 females) adolescents (representing all those available to the cohort study in 1995; Singhal, unpublished data) and the effect of prepubertal growth on the genotype difference in the age at take off investigated. In girls TO was delayed by 1.7 years in SS disease and this genotype difference was significantly related to genotype differences in height (partial correlation $r = -0.23$, $p = 0.03$; Analysis of variance) and weight at age 8 years ($r = -0.35$, $p < 0.001$) but not to the sum of skinfolds ($r = -0.21$, $p = 0.06$), HbF ($r = -0.12$, $p = 0.24$) or Hb levels ($r = -0.06$, $p = 0.54$) at the same age. For boys the delay in TO in SS disease (1.0 year) was related to genotype differences in the sum of skinfolds ($r = -0.29$, $p = 0.01$) but not to height ($r = 0.01$, $p = 0.90$), weight ($r = -0.12$, $p = 0.29$), HbF ($r = 0.00$, $p = 0.99$), or Hb levels ($r = -0.09$, $p = 0.35$) at age 8 years. These observations were consistent with the hypothesis that better prepubertal growth favoured less delay in the onset of adolescent growth and suggested that investigation of the determinants of prepubertal growth could shed light on the mechanisms of growth failure in SS disease.

Summary

To investigate the timing and pattern of the adolescent growth spurt, longitudinal observations of height from the Jamaican cohort study were fitted to a mathematical model of growth (Preece-Baines model 1). Compared to AA controls, the onset of the adolescent growth spurt in SS children was delayed by 1.4 years and the age at peak height velocity delayed by 1.6 years and there was no sex difference in this delay. There was no genotype difference in the attained height by age 17.9 years suggesting a good prognosis for final height in SS children. The growth spurt was not delayed in SC disease.

The age at menarche in girls with SS disease (mean, SD: 15.4, 1.3 years) was significantly later than girls with SC disease (13.7, 1.7 years) and those with AA haemoglobin (13.1, 1.3 years) but these genotype differences were no longer significant after controlling for the delay in the adolescent growth spurt. The normally co-ordinated but delayed onset of adolescent growth suggest that factors determining the onset of the pubertal growth spurt, including factors affecting prepubertal growth are likely to be the major determinants of the overall growth impairment in SS disease.

FACTORS AFFECTING PREPUBERTAL GROWTH

Optimum prepubertal growth is a major determinant of the onset of the adolescent growth spurt (Tanner 1962) and factors impairing prepubertal growth are likely to retard adolescent growth and development. Impaired prepubertal growth is common in children with SS disease but the factors responsible are unknown. The present study therefore assessed the growth of cohort SS children over the first 10 years in relation to haematological indices, socioeconomic status and clinical course.

Subjects and Methods

All subjects participated in the Jamaican cohort study of SS disease as previously described and the present report is confined to SS children with observations on growth for at least the first 7 years. Of the 315 SS children detected at birth, 4 failed to be recruited to the study, 48 died and 4 emigrated before their 7th birthday. A further 18 (16 with cerebro-vascular accidents, 1 with mental retardation and 1 with systemic lupus erythematosus) were excluded because other factors may have influenced growth. The final study group included 241 children, although data in all children were not available for all analyses.

Heights and weights were measured at 3 month intervals coinciding with birthdays by methods previously described. The selected indices of growth were the attained height, weight, weight/height and weight/height^2, and their relationship with haematology was analysed at ages 3,5, and 7 years although only the more complete data available at 7 years are presented. Prepubertal growth was also depicted as the increment in height between 3-9 years, an age range selected because of the less reliable growth measurements and the more rapid age related changes in steady-state haematology before age 3 years and the confounding effects of the pubertal growth spurt after age 9 years. Computer generated curves

of height against age were used to identify and appropriately edit outlying values as previously described. Socioeconomic data were available only at age 8 years.

Haematological indices, and HbF levels were measured as previously described, F-reticulocyte and F-cell counts measured in 63 SS patients by the technique of Dover *et al* (1978a), and the number of alpha globin genes determined in 232 children available to the study after 1976. Only haematological indices taken during clinically well visits, excluding any values for 3 months after a blood transfusion were used in analyses. Socioeconomic status and morbidity data were collected as previously described. Disease severity was assessed as the number of specific clinical events before the age of 7 years and the distribution of number of events was grouped into approximate quartiles (painful crises: 0 [n=97], 1 [n=58], 2-4 [n=58], $\geq$ 5 [n=28]; hospital admissions: 0 [n=71], 1 [n=61], 2-3 [n=68], $\geq$ 4 [n=41]; sick visits: 0-7 [n=57], 8-12 [n=61], 13-18 [n=61], > 19 [n=62]; acute chest syndrome: 0 [n=75], 1 [n=61], 2-3 [n=60], and $\geq$ 4 [n=45]). Episodes of acute splenic sequestration were categorised as 0 [n=175], 1 [n=33], $\geq$2 [n=33] events. The contribution of parental height to linear growth in SS disease could not be assessed since protocols did not collect data on parental height as this would have been incomplete because of the high frequency of single parent or adopted families.

Statistical Analysis

Statistical associations between growth, haematological indices, and SES were initially examined by correlation coefficient analysis and, when appropriate, by partial correlation analysis to control for potential confounders. Comparison of growth between morbidity groups and sex were assessed by analysis of variance. The distribution of total HbF, reticulocytes, F-cells, and F-reticulocytes were skewed and were transformed ($\log_{10}$[HbF+1], $\log_{10}$[reticulocytes], $\log_{10}$[F-reticulocytes] and $\log_{10}$[F-cells/F-reticulocytes]) for parametric analysis.

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Results

Gender Differences

At age 7 years there were no significant sex differences in height, weight, socioeconomic score (Table 4) or the frequency of any of the five indices of morbidity (Table 5). There were no significant gender differences in steady-state HbF level in this age group (p=0.48 using Mann-Whitney U test) or in the number of subjects (6 boys, 1 girl) with HbF levels below 1% (which is the 5th centile for the HbF distribution of the sexes combined), (p=0.15 using Fishers exact test). Weight/height and weight/height^2 were greater in boys and steady-state Hb levels higher in girls.

Table 4

Distribution of Growth, Haematology, Morbidity, and Socioeconomic Indices

in SS Children at Age 7 Years

(comparison by Mann Whitney U-test)

	Males			Females			
	n	median	(range)	n	median	(range)	p
Height (cm)	113	117.0	(104.5-127.4)	106	117.9	(106.7-131.3)	0.39
Weight (kg)	113	19.7	(14.4-24.7)	105	19.1	(14.1-25.7)	0.075
Weight/height (x100)	113	16.6	(13.8-19.6)	105	16.2	(13.0-20.3)	0.007
Weight/height^2(x1000)	113	1.4	(1.21-1.62)	105	1.4	(1.16-1.60)	<0.001
Haemoglobin (g/L)[1]	93	74.0	(56-103)	80	80.0	(57-106)	0.006
Haemolysate HbF (%)	71	5.9	(0.3-26.2)	65	6.1	(0.9-21.0)	0.48
Socioeconomic score (maximum 18)	92	12.0	(2-18)	91	11.0	(1-18)	0.12

[1]Haematology at nearest steady state visit but within 6 months of 7th birthday

Table 5

Frequency Distribution of Indices of Morbidity in 7 year old SS Children

(n = 241; 126 M, 115 F)

		Group 1[a] No (%)		Group 2 No (%)		Group 3 No. (%)		Group 4 No. (%)	
Painful Crises	M	56	(44)	31	(25)	25	(20)	14	(11)
	F	41	(36)	27	(29)	33	(29)	14	(12)
Hospital Admissions	M	34	(27)	32	(25)	41	(33)	19	(15)
	F	37	(32)	29	(25)	27	(23)	22	(19)
Sick Visits	M	27	(21)	29	(23)	37	(29)	33	(26)
	F	30	(26)	32	(28)	24	(21)	29	(25)
Acute Chest	M	38	(30)	32	(25)	29	(23)	27	(21)
Syndrome	F	37	(32)	29	(25)	31	(27)	18	(16)
Acute Splenic	M	92	(73)	18	(14)	16	(13)	-	-
Sequestration	F	83	(72)	15	(13)	17	(15)	-	-

M= Males; F= Females

[a] Groups 1-4: Number of clinical events up to age 7 years: 0, 1, 2-4, >5 painful crises; 0, 1, 2-3, >4 hospital admissions; 0-7, 8-12, 13-18, >19 sick visits; 0, 1, 2-3, >4 episodes of acute chest syndrome; and 0, 1, >2 episodes of acute splenic sequestration.

No significant sex difference for distribution of any index of morbidity.

Growth and Haematology.

Heights and weights at ages 3, 5 and 7 years correlated with steady-state HbF levels in boys but not in girls (results at 7 years in Table 6). Scatter plots of height against HbF did not support the notion of 'cut off' level at which high HbF has a protective effect on height. Weight/height, and weight/height^2 correlated with HbF in boys at age 7 years, and weight/height correlated with HbF in boys at age 3, but not in girls at any age. Height, weight, weight/height, and weight/height^2 correlated with Hb concentration in boys at age 5 and 7 years but not at 3 years, and not at any age in girls. Gender differences in the correlation coefficients existed for the relationships between Hb and weight and weight/height but not for any of the other correlations.

Table 6				
Relationship of some Growth Indices at age 7 Years with Steady-state Total Haemoglobin and Fetal Haemoglobin (Product Moment Correlation Coefficients)				
		Height	Weight	Weight Height	Weight Height^2
Boys					
Haemoglobin	r	0.30	0.36*	0.35*	0.25*
	n	88	88	88	88
	p	0.005	<0.001	0.001	0.02
Log$_{10}$ (HbF+1)	r	0.34	0.40	0.38	0.25
	n	68	68	68	68
	p	0.005	<0.001	0.002	0.043
Girls					
Haemoglobin	r	0.03	-0.02*	-0.04*	-0.06*
	n	76	75	75	75
	p	0.80	0.86	0.74	0.62
Log$_{10}$ (HbF+1)	r	0.18	0.21	0.17	0.06
	n	62	61	61	61
	p	0.16	0.10	0.18	0.67
* significant gender difference in correlation coefficients					

The increment in height from 3-9 years correlated significantly with steady-state Hb concentration at age 7 years (r=0.33, p=0.006) and almost with HbF (r=0.27, p=0.056) in boys but not in girls (r=0.01, p=0.94 and r=0.20, p=0.17 respectively). In boys partial correlation analysis of height and weight at 7 years on Hb, and HbF levels suggested that HbF accounted for the effect of Hb since the latter was no longer significant after controlling for HbF. For weight/height, weight/height^2 and height increment it was not possible to identify the more important variable since associations with both Hb and HbF became not significant after controlling for the other.

Attained height at 7 years correlated significantly with the percentage of F-cells and F-reticulocytes (Table 7) in boys and that for F-reticulocytes just reached failed to reach significance for girls. The ratio of F-cells to F-reticulocytes (a measure of F-cell enrichment, Dover *et al*, 1978b) was not significantly related to height at 7 years in either sex and there were no significant gender differences in these correlations. No significant relationships were apparent between attained height or height increment and mean cell volume, reticulocyte count, or number of alpha globin genes in either sex (results not given).

		F Cells	Log$_{10}$ (F retics)	Log$_{10}$ (F cells/F-retics)
Boys	r	0.44	0.46	-0.23
	n	38	40	38
	p	0.006	0.003	0.16
Girls	r	0.17	0.39	-0.33
	p	26	26	26
	n	0.42	0.05	0.10

Table 7

Relationship of Attained Height at age 7 Years

with F-cells, F-reticulocytes, and F-cell/F-reticulocyte ratio
(product moment correlation coefficient)

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Growth and Socioeconomic Status

Socioeconomic scores did not correlate with any growth index at age 7 years or with height increment between age 3-9 years in either sex (correlations between -0.11 and 0.20).

Growth, Haematology and Morbidity

Neither height nor weight were related to the number of hospital admissions (Table 8) or any of the 5 indices of morbidity except for a lower mean weight in female subjects with 0-7 sick visits (p=0.02). A low HbF concentration was significantly associated with an increased number of sick visits (p=0.008), hospital admissions (p<0.001) and episodes of acute splenic sequestration (p=0.03) but associations between HbF level and episodes of acute chest syndrome (p=0.054) or painful crises (p=0.053) just failed to reach significance. However the relationship between HbF and painful crises showed a significant sex interaction and was strongly significant for boys (p=0.003) but not girls (p=0.20) (Table 9). Low Hb concentrations were associated with an increased number of hospital admissions (p<0.001) and episodes of acute chest syndrome (p=0.03) but no significant associations occurred between Hb and the number of painful crises (p=0.65), sick visits (p=0.55) or episodes of acute splenic sequestration (p=0.09). There were no gender differences in any association between Hb concentration and index of morbidity.

A higher SES was significantly associated with a greater number of sick visits (p=0.002) and almost with the episodes of acute chest syndrome (p=0.051), but not with other morbidity indices.

Table 8

Relationship Between Number of Hospital Admissions, Haematology and Growth

Figures are mean (SD) except for fetal haemoglobin which is median (range)

	Sex	0	1	2,3	4+	p[1]
			Number of hospital admissions			
Height	M	117.6 (5.6)	116.9 (4.2)	117.4 (5.5)	116.3 (4.2)	0.82
(cm)	F	119.1 (4.6)	116.7 (4.8)	116.2 (5.2)	119.0 (5.5)	0.06
Weight	M	19.7 (2.2)	19.7 (2.4)	19.6 (2.4)	19.1 (1.4)	0.83
(kg)	F	19.6 (2.3)	18.6 (2.1)	18.5 (1.5)	19.5 (1.9)	0.08
Hb	M	82 (11)	76 (10)	72 (11)	71 (10)	0.005
(g/L)	F	84 (12)	80 (8)	76 (9)	76 (11)	0.03
HbF	M	7.6	6.6	3.3	3.3	
(% Hb)		(0.7-26.2)	(1.1-19.3)	(0.3-18.3)	(0.5-12.6)	0.03
	F	8.0	8.0	4.8	3.8	
		(2.9-16.7)	(2.4-21.0)	(1.0-10.6)	(0.9-8.0)	0.0005

[1]Analysis of Variance

Table 9

Relationship of Painful Crisis Frequency, Haemoglobin and Haemoglobin F

Figures are mean (SD); (Analysis of Variance)

		0 Episodes	1 Episode	2-4 Episodes	>5 Episodes
Hb	M	79 (12)	73 (10)	73 (10)	73 (09)
(g/L)	F	76 (09)	80 (10)	82 (10)	85 (10)
Log_{10} (HbF)	M	0.94 (0.27)	0.75 (0.36)	0.66 (0.35)	0.49 (0.19)
(% Hb)	F	0.80 (0.25)	0.98 (0.18)	0.80 (0.31)	0.86 (0.22)

Missing Data

The growth of 48/315 patients with SS disease who died before the age of 7 years was assessed by calculating standardised scores (z scores) for height (relative to the growth of the whole cohort) at the age of their last assessment. The mean (SD) z score of patients that had died, -0.24 (1.34), was not significantly different from zero, suggesting that children remaining in the study at age 7 years were representative of those diagnosed at birth.

Of the 241 children theoretically eligible for the analysis at age 7 years, values at 7 years ($\pm$ 3 months) were not available for height in 22 (9%; 13 male, 9 female), steady-state Hb in 68 (28%; 33 M, 35 F), steady-state HbF in 105 (44%; 55 M, 50 F). However Hb and HbF values calculated as the mean of all steady-state values from age 5 years did not differ substantially in patients with and without steady-state data at age 7 years and all differences were not significant, suggesting that bias was not introduced by exclusion of these subjects. Furthermore the mean heights of patients with or without steady-state HbF and Hb concentrations at age 7 years were similar although there was a trend for patients with steady-state Hb and HbF levels at age 7 years to be approximately 0.7 kg heavier than those without such data. This observation was not unexpected since patients sick at their 7 year visit would not have their hematology included in the analysis and would probably be lighter as a result of their illness. The height, Hb, and HbF levels (at age 7 years) of the subset of patients with F-cell data did not differ from subjects without such data.

Discussion

Growth retardation in a chronic condition such as SS disease may have many different aetiologies. Anaemia, recurrent infections and other aspects of chronic ill health are assumed to contribute but there is a dearth of data. The present study has analysed several indices of growth between age 3-9 years and found significant

relationships with haemoglobin and fetal haemoglobin levels, but no contributions from indices of morbidity or socioeconomic status.

The lack of effect from socioeconomic status was surprising since low SES is well established as a cause of poor growth. This may reflect the relatively imprecise nature of the socioeconomic scoring system employed in the present study (Alleyne et al, 1979) which documented water supply and sewage services but leant heavily on provision of services such as electricity, telephone, and appliance use. This is known to underestimate the SES of people in rural areas where nutrition may be excellent but electricity and telephone services unavailable. The social structure of many Jamaican families does not allow the use of paternal income as a reliable indicator of socioeconomic status and even maternal income may be erratic and inconsistent. It seems likely that the lack of an apparent effect of SES may be attributable to a relatively insensitive indicator of SES in this community.

The present study searched for haematologic correlates of growth delay in SS disease and found no relationship with mean cell volume, reticulocyte count or the presence of alpha thalassaemia. Total haemoglobin and HbF levels were significantly related to attained height and weight at age 5 and 7 years and with height increment from age 3-9 years but these relationships were confined to boys and no trend was apparent in girls. Further analysis suggest that the apparent effect of total haemoglobin was mediated through an effect of HbF.

The uneven intracellular distribution of HbF in SS disease implies that red cells may contain large or small amounts of HbF, cells with low HbF levels being more prone to sickling and hence earlier removal from the circulation. Cells with high HbF levels persist for longer and thus cell selection increases haemolysate HbF level. Theoretically, elevated HbF may result from increased production (measured by F-reticulocyte counts; Dover et al, 1978b) or from cell selection (measured by the F-cell to F- reticulocyte ratio; Dover et al, 1978b) without increased production. Since these two mechanisms have different implications, the

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relationship was further examined in a subgroup of subjects in whom both F-cell and F-reticulocyte counts were available. This showed that only the F-reticulocyte count and not the F-cell to F-reticulocyte ratio was related to height at 7 years. Even in females where no significant relationship between growth and haemolysate HbF or F-cells was apparent, the relationship with F-reticulocyte count reached significance. Since the F-reticulocyte count in SS disease is characteristic of an individual (Dover *et al*, 1978b) these observations suggest that the individual level of HbF production is the factor related to better growth.

Possible mechanisms whereby high HbF levels influence growth remain unclear but symmetrical growth retardation in SS disease argues against a local effect of vaso-occlusion on individual growth plates but rather for a centrally mediated decrease in height velocity. By reducing erythropoietic activity high HbF levels could reduce the competition from erythropoiesis for amino acids and calories essential for growth. Alternatively, the greater clinical severity in patients with low HbF levels (Bailey *et al*, 1992) could retard growth, but although low HbF concentrations were associated with greater morbidity in the present study, there was no relationship between morbidity indices and growth failure to support such a mechanism. It is also possible that indices such as painful crises, acute chest syndrome and acute splenic sequestration are of too short duration to affect growth or that faltering growth is obscured by catch up growth during asymptomatic periods. The hypothesis that a low HbF concentration affects growth in SS disease by increasing erythropoiesis could further be investigated by assessing the growth of SS children who have a high rate of erythropoiesis. A simple measure of erythropoietic activity in large numbers of patients was not available, but the marked increase in red cell turnover in patients with chronic hypersplenism provided a useful model of high erythropoietic activity. As many hypersplenic SS children required splenectomy for recurrent attacks of acute splenic sequestration the potential benefit of reducing erythropoiesis on growth could also be investigated.

Summary

The present study has analysed several indices of growth in SS children between 3-9 years and found significant relationships with haemoglobin and fetal haemoglobin levels, but no contributions from other haematological indices, indices of morbidity or socioeconomic status.

Height increment between 3 and 9 years of age correlated with total Hb and HbF in boys but not girls. Attained height and weight at age 7 years correlated with Hb and HbF in boys only and partial correlation analysis suggested that the contribution of total Hb was accounted for by the effect of HbF. Further analysis indicated that attained height correlated with F-reticulocyte count (a measure of HbF production) but not with the ratio of F cells to F-reticulocytes (a measure of F-cell enrichment).

A high HbF level in boys with SS disease is associated with greater linear growth generating the hypothesis that low HbF levels in SS boys may increase haemolysis and metabolic requirements for erythropoiesis and therefore put boys at greater risk of poor growth. The effect of erythropoiesis on growth in SS disease could be investigated in models where there is a marked increase red cell turnover such as chronic hypersplenism. This complication produces a sustained metabolic stress that could impair compensatory catch up growth between episodes of acute illness and also has the advantage of producing a marked reduction in erythropoietic activity after splenectomy.

HYPERSPLENISM AND GROWTH

Chronic hypersplenism markedly increases haemolysis and the resulting erythropoietic expansion is likely to have a high metabolic cost. Protein turnover consistently falls following splenectomy for hypersplenism (Badaloo *et al*, 1991) and preliminary observations suggest that growth slows during hypersplenism and accelerates following splenectomy (Emond 1987). The hypothesis that hypersplenism imposes a high metabolic load which competes with the requirements for normal growth was tested in children with hypersplenism and SS disease by observations of growth before and after splenectomy.

Subjects and Methods

Review of all records of patients attending the sickle cell clinic (cohort and non cohort groups) identified 32 children (13M, 19F) with SS disease aged 1-11 years who had splenectomy for hypersplenism between 1/1/73 and 31/12/91. Children below 1 year of age were excluded since reliable growth data are difficult to obtain at this age and those aged over 11 years were excluded to avoid the possible confounding effects of the pubertal growth spurt. Median age at splenectomy was 4.7 years (range: 1.4 - 9.3).

Haematological indices including Hb concentration, MCV, and MCHC were measured in children 3 months before splenectomy (while untransfused) and 3 months post-splenectomy (when transfused blood had disappeared). Patients were not given regular blood transfusion treatment for hypersplenism and were transfused only if deemed necessary at the time of the splenectomy. Chronic hypersplenism was arbitrarily defined as a spleen of 4 cm or more below the left costal margin, Hb <60 g/L, reticulocyte counts >15%, and platelet count <200 x 10^9/L, all recorded on at least 2 occasions 3 months or more apart.

Height and weight were measured at 3 month intervals as previously described and the nearest available data used for computing velocities over three 6

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month periods (6 months pre-splenectomy, 6 months post-splenectomy, 6-12 months post-splenectomy). Height and weight velocities were expressed over one year intervals by multiplying the actual measured increments by an appropriate factor. Height and weight at time of splenectomy were available before operation in 22 patients and immediately after operation in 10. Weight velocities, pre- and post-splenectomy were also calculated taking account of splenic weight (median 0.32 kg, range 0.07 - 0.78 kg). Standardised scores (z scores) for height, weight, weight/height, height velocity and weight velocity in hypersplenic patients were calculated using age and sex specific standards derived from the cohort study of 200 SS subjects.

The statistical significance of the changes in height and weight velocity, height, weight, and weight/height (expressed in original units or as z scores) and haematology before and after splenectomy were assessed using the paired t-test (which excludes subjects without data both pre- and post-splenectomy). The one sample t-test was used to assess whether standardised scores for growth indices were from a population with a mean of zero.

Results

The mean height velocity of hypersplenic children in the 6 months before splenectomy was 7.0 (SD 3.1) cm/yr. (95% CI: 6.0 to 8.1) compared to 9.2 (SD: 2.8) cm/yr. (95% CI: 8.2 to 10.2) in the 6 months following operation, representing a mean increase of 2.2 cm/yr (95% CI: 0.7 to 3.7). This acceleration in growth was not sustained into the period 6-12 months following splenectomy when the mean height velocity fell to 6.6 (SD: 3.1) cm/yr. (95% CI: 5.5 to 7.8). The change in height velocity associated with splenectomy relative to values observed in control SS children are shown in Figure 3a (males) and Figure 3b (females) and as standardised scores in Table 10. Height velocities (10th, 50th and 90th centiles) for a cohort of 200 Jamaican SS children, calculated over 12 month intervals are also given for comparison in Figure 3a and 3b.

Table 10

Standardised Scores (z scores) for Growth Indices Before and After Splenectomy

	Before operation	At operation	Postoperative (1)	Postoperative (2)	Comparison of columns 1-4					
	n Mean (SD)	n Mean (SD)	n Mean (SD)	n Mean (SD)	1v2	1v3	1v4	2v3	2v4	3v4
Height Velocity	32 -0.01 (1.83)	-	32 1.82 (1.75)+++	29 0.10 (2.46)	-	***	NS	-	-	**
Height	32 -0.07 (1.03)	32 -0.13 (0.94)	32 0.16 (1.01)	29 0.27 (1.01)	NS	***	**	***	***	NS
Weight Velocity	31 -0.32 (2.46)	-	31 0.12 (2.47)	28 0.51 (2.33)	-	NS	NS	-	-	NS
Weight	32 0.30 (1.18)	31 0.24 (0.98)	32 0.33 (1.16)	29 0.39 (1.14)	NS	NS	NS	NS	NS	NS
Weight/Height	31 0.43 (1.20)	31 0.41 (0.92)+	32 0.30 (1.12)	28 0.49 (1.18)+	NS	NS	NS	NS	NS	NS

− Not applicable

Comparison using paired t-test; *** p<0.001, ** p<0.01, * p<0.05;   One sample t-test; +++ p<0.001, + p<0.05.

Height and weight velocities were measured over 0-6 months pre-splenectomy, 0-6 months postoperatively (1), and 6-12 months postoperatively (2).

Weight velocities calculated without consideration of splenic weight.

NS= Not Significant

Figure 3a and 3b:
Height velocity for boys (3a) and girls (3b) over 6 month intervals before and after splenectomy for hypersplenism connected by a straight line in individual boys and girls.
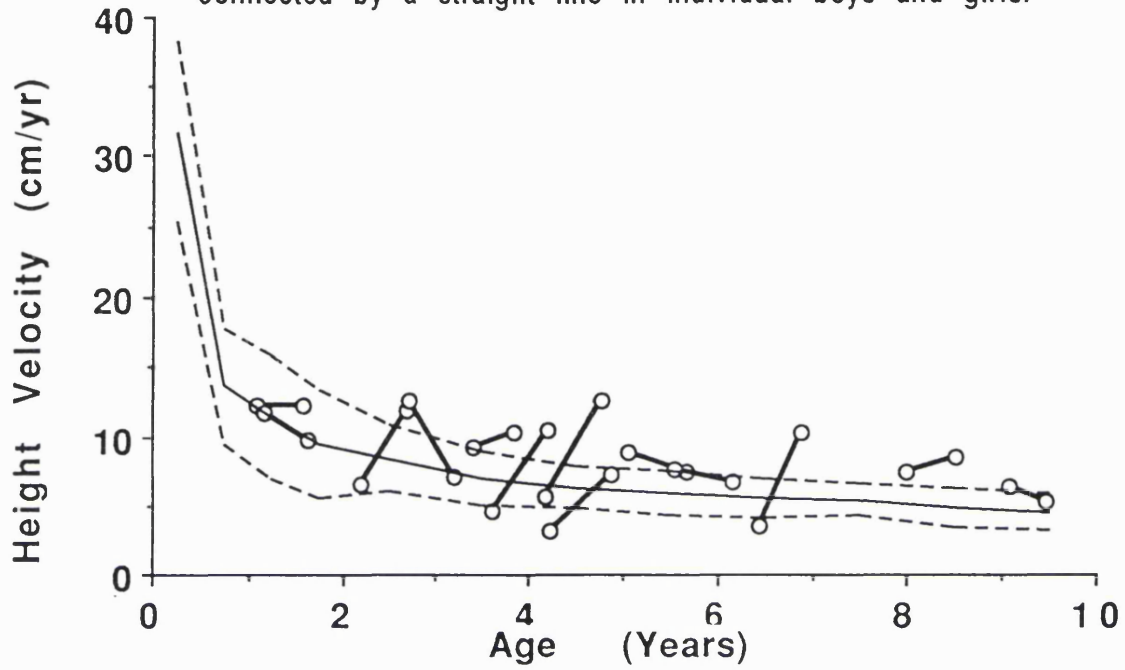
Figure 3b Girls
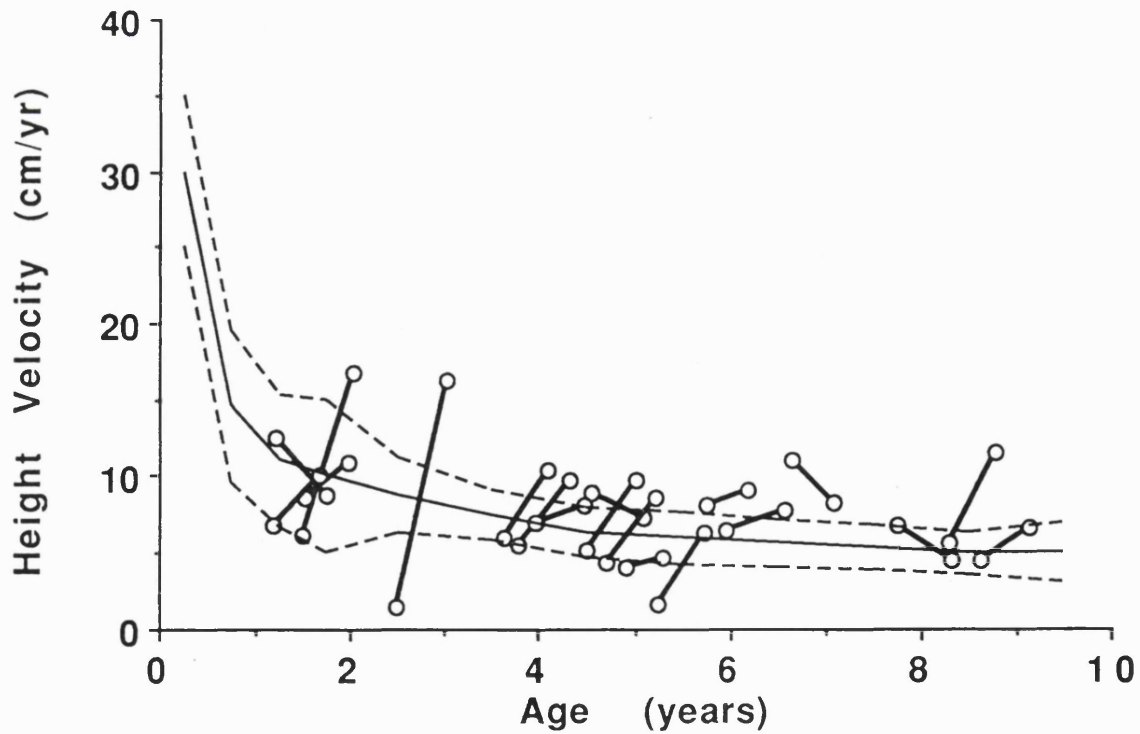
The mean weight velocity in the 6 months before operation was 1.5 (SD: 2.0) kg/yr. (95% CI: 0.8 to 2.2) compared to 2.0 (SD: 2.0) kg/yr (95% CI: 1.2 to 2.7) in the 6 months following splenectomy, a mean increase 0.5 kg/yr. (95% CI: -0.7 to 1.6) (p=0.4).   Comparing weight velocities that had been adjusted for splenic weight showed a mean increase of 0.6 kg/yr. (95% CI: -0.6 to 1.8) (p=0.3) after splenectomy. Weight velocities expressed as standardised scores (Table 10) also failed to show significant increases following splenectomy. Accelerated linear growth following splenectomy for hypersplenism occurred despite normal z scores for height and weight 6 months before and at splenectomy, and normal z scores for weight velocity and height in the 6 months following operation (Table 10).

Body mass index (BMI, calculated as weight/height^2) of hypersplenic patients at splenectomy (mean, SD: 15.2, 1.2 kg/m^2; range: 13.3 - 18.0) was similar to that 6 months before splenectomy (mean, SD: 15.4, 1.5 kg/m^2; range 13.0 - 19.4) (p=0.2) and the ranges were similar to the BMI of the cohort of 200 SS children aged 1 to 10 years (range: 11.1 - 19.9 kg/m^2). Six months post-operatively, the BMI had fallen (mean, SD: 14.7, 1.2 kg/m^2; range: 12.9 - 17.2) (p=0.004) and remained low 12 months following splenectomy (mean, SD: 14.8, 1.3 kg/m^2; range: 13.1 - 17.2).

Striking haematological changes followed splenectomy for hypersplenism (Table 11) with significant increases in total Hb, haematocrit, MCHC, RBC, and platelet count and a fall in reticulocyte count. Since both height velocity and haematological indices change following splenectomy, the relationship of change in height velocity to pre-splenectomy haematological indices and to degree of change in these indices was examined to search for possible correlates. The increase in height velocity post-operatively correlated negatively with the change in MCHC and significant or borderline significant correlations occurred with pre-splenectomy haemoglobin, MCHC, MCV, and RBC (Table 12).

Table 11

Changes in Haematology After Splenectomy For Hypersplenism

	Before-splenectomy (n=29) mean	(SD)	After-splenectomy (n=27) mean	(95% CI)	p value (paired t test)
Hb (g/L)	51	(12)	83	(77 to 88)	<0.001
Haematocrit (%)	16.7	(3.7)	25.1	(23.4 to 26.9)	<0.001
MCHC (g/L)	309	(32)	332	(316 to 348)	0.007
RBC (x 10^{12}/L)	2.0	(0.5)	3.1	(2.9 to 3.3)	0.001
MCV (fL)	+		+		0.06
MCH (pg)	26.3	(4.1)	27.0	(25.4 to 28.6)	0.4
Reticulocytes (%)	20.0	(8.3)	8.7	(5.3 to 12.0)	0.001
Platelets (x 10^9/L)	231	(109)	579	(483, 651)*	<0.001

+Variable not normally distributed: median of MCV before splenectomy is 85 fL (range: 69 - 117) and after splenectomy 82.0 fL (95% CI: 79 to 85.1). * n=21

Table 12

Correlation Between Change in Height Velocity and

Haematological Indices in SS Patients with Hypersplenism

	Haematology before-splenectomy (n=29) Correlation Coefficient	p	Change in haematology (n=27) Correlation Coefficient	p
Haemoglobin	0.35	0.06	-0.22	0.28
Haematocrit	0.21	0.28	0.00	0.99
MCHC	0.38	0.04	-0.47	0.01
RBC	0.35	0.06	0.08	0.68
MCV	-0.35	0.06	0.02	0.93
MCH	-0.10	0.62	-0.32	0.10
Reticulocytes	0.00	0.99	-0.05	0.81
Platelets	-0.07	0.72	0.31*	0.18
HbA$_2$	0.17	0.37	–	–
HbF	0.04+	0.85	–	–

+In transformation used;   * n = 21;
– HbA$_2$ and HbF not routinely measured post-splenectomy

[handwritten: explain?]

Multiple regression analysis confirmed MCHC as the only independent correlate of the pre-splenectomy haematology with change in height velocity following splenectomy. Change in height velocity did not correlate with pre-splenectomy serum iron (r= -0.09, p=0.63), iron binding capacity (r=0.32, p=0.09), transferrin saturation (r= -0.14, p=0.46), or serum folate level (r=0.12, p=0.58).

Discussion

Hypersplenism is associated with excessive red cell destruction in SS disease with mean red cell life spans as short as 2 days (Rossi *et al*, 1964). In the face of this haemolysis, haemoglobin levels of 30-60 g/L can only be maintained by marked erythropoietic expansion and consequently increased demands of amino acids and calories that may compete with demands for growth. The normal mean standardised scores for height and height velocity (compared to a cohort of SS children) suggest that SS patients with hypersplenism adapt to the increased erythropoietic demands and maintain normal growth. Splenectomy for hypersplenism was associated with an improvement in haematological indices and an increase in height velocity. The need to document height velocity over periods as short as 6 months inevitably leads to greater variability as evidenced by the greater than expected spread in pre-splenectomy values apparent in Figures 3a and 3b. Despite this source of inaccuracy, there was a significant increase in height velocity, suggesting that processes compensating for hypersplenism prior to splenectomy produce a temporary growth spurt once the excessive erythropoietic stress is removed. Similar observations of accelerated height velocity have also been made in Crohn's disease (Lipson *et al*, 1990) and Trichuris infection (Cooper *et al*, 1990) following treatment of the underlying pathology.

[handwritten: turnover not loss (leukaem)]

[handwritten: Why short-lived?]

[handwritten: Sustained]

The increase in weight velocity following splenectomy for hypersplenism was not significant. Adjusting for splenic weight had little effect on the change in weight velocity after surgery, although such adjustment must be very inaccurate, splenic weight being influenced by random factors such as the degree of

sequestration at the time of operation and the interval between clamping of the splenic artery and vein. The increase in height velocity following splenectomy clearly exceeds any increase in weight velocity, contrasting with the effect of nutritional supplementation of severely malnourished children (Walker and Golden, 1988) in whom an increase in weight velocity precedes an increase in height velocity. This suggests that the accelerated linear growth is not simply consequent on a general improvement in nutrition but that hypersplenism produces specific nutritional demands. The fall in body mass index in the 6 months following splenectomy is consistent with this observation and argues against a general calorie deficiency (Heyman *et al*, 1985) but in favour of a specific defect such as the marginal availability of glycine in this condition (Jackson 1984).

The failure of the increase in post-splenectomy height velocity to correlate with an improvement in haematological indices was surprising and suggests that the acceleration in growth was not related to any of these factors. Changes in height velocity post-splenectomy correlated positively with pre-splenectomy MCHC values and negatively with changes in MCHC levels. These observations and the borderline significant correlations with pre-splenectomy haemoglobin levels and red blood cell count suggest that children able to maintain higher haemoglobin production in the face of a greatly increased erythropoietic stress were better equipped for a growth spurt following splenectomy.

Summary

Splenectomy for chronic hypersplenism was followed by highly significant changes in haematological indices and an increase in height, but not weight, velocity. The increase in height velocity was qualitatively similar to that observed in the Trichuris dysentery syndrome following treatment and differed from the increases in height and weight velocity following nutritional supplementation of severely malnourished children. These observations were therefore consistent with the hypothesis that increased erythropoiesis impairs growth in SS disease.

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DETERMINANTS OF GROWTH: DISCUSSION

Analysis of height data from the Jamaican cohort study suggested that adolescent growth and sexual maturation are delayed in SS children but final height is normal. A mixture of genetic and environment factors affect the timing of the adolescent growth spurt (Tanner 1962) but similar birth weights, socioeconomic status and physical environment in cohort SS and AA children suggest that these variables did not contribute to delayed growth in Jamaican SS children. Furthermore, the general and symmetrical growth retardation in SS children argues against localised vaso-occlusive crises affecting the growth of long bones and the lack of a similar delay in SC children, despite their frequent vaso-occlusive events, supports this hypothesis. Nutrition is considered to be the strongest environmental influence on growth (Eveleth 1985) and the similarity in growth pattern of SS children to chronically malnourished AA children (Kulin *et al*, 1982; Eveleth 1985; Tanner 1962) is consistent with the hypothesis that nutritional factors retard growth in SS disease.

Chronic malnutrition retards sexual and skeletal maturation and delays the onset of the adolescent growth spurt. Height gain during adolescence tends not to be affected, any deficit in final height resulting from suboptimal prepubertal growth (Eveleth 1985). Children with poor prepubertal growth have a later and prolonged growth spurt (Tanner 1962) that allows a longer growth period to compensate for adverse environmental circumstances. In severe malnutrition, however the longer growth period may be insufficient to allow catch up in height and final height is reduced. A similar growth pattern and deficits in height and skinfold thickness are consistent with suboptimal nutrition delaying growth in SS children although normal final height in SS adults suggests either a moderate environmental insult or a delay in epiphyseal fusion (of unknown mechanism) that allows catch up in final height.

Factors affecting growth in SS disease emerge early in infancy and 11% of girls and 18% of SS boys in the cohort study were stunted (height less than mean minus 2 standard deviations of the NCHS growth reference) by the age of 2 years (Singhal, unpublished observations) compared to less than 10% prevalence in the Jamaican population (Keller 1988). This early growth retardation is likely to impair the whole growth pattern and the greater delay in adolescent growth of poorly growing prepubertal SS children was consistent with this hypothesis.

The determinants of prepubertal growth in SS disease are unknown but could include recurrent episodes of ill health or abnormal haematology. In the present study, attained height at age 7 years and the increment in height between 3 and 9 years were not related to morbidity or socioeconomic status and only high haemoglobin F emerged as a protective factor against growth delay. The ameliorating effect of HbF on the clinical course of SS disease is widely recognised (Stevens et al, 1981; Bailey et al, 1992) and higher HbF concentrations in children from the cohort study were associated with fewer hospital admissions, sick visits to the clinic, episodes of acute splenic sequestration and painful crises. High HbF levels could therefore prevent growth delay by reducing morbidity although there was no relationship between morbidity indices and growth failure to support such a mechanism. Alternatively HbF could reduce the metabolic demands of a high erythropoietic rate so favouring better growth.

Unexpectedly, the protective effect of high HbF against frequent painful crisis and poor growth was confined to boys. Observations that the beneficial effect of HbF on manifestations of SS disease are confined to males have also been previously reported for age at first occurrence of painful crises, acute splenic sequestration and dactylitis (Bailey et al, 1992), for abnormal body habitus (Ashcroft and Serjeant, 1972), skeletal retardation (Serjeant and Ashcroft, 1973), proliferative sickle retinopathy (Hayes et al, 1981) and avascular necrosis of the femoral head (Hawker et al, 1985). Genotype differences in anthropometric indices and nutrition intake were also confined to males in a Nigerian study

(Modebe and Ifenu, 1993). One possible mechanism of this sex related effect is that the relationship is created by subjects with very low HbF concentrations who are predominantly male (Bailey *et al*, 1992) but there was no significant sex difference in the overall distribution of HbF concentration or in the number of children with very low HbF concentrations in the present study. Another possibility is that a low HbF level further increases erythropoietic and metabolic activity which may already be greater in SS boys than girls, and this hypothesis is supported by the greater resting metabolic rate (Singhal *et al*, 1993a) irreversibly sickled cell counts (a determinant of haemolysis) (Mason *et al*, 1982), serum transferrin receptor levels (a measure of erythropoietic activity) (Singhal *et al*, 1993b) and poorer growth in males (Phebus *et al*, 1984). High HbF may reduce the metabolic demands of bone marrow so protecting against both avascular complications and poor growth in boys. Future analyses should therefore recognise that the factors affecting growth in SS disease may differ between males and females.

Gender differences also occurred in the pattern of growth in SS children, with boys suffering more growth retardation than girls. In the cohort study adolescent growth delay was greater in boys although small and unequal numbers of boys and girls limited interpretation of these data. These observations raise the possibility that the growth of SS boys is more susceptible to adverse environmental circumstances or alternatively, additional mechanisms contribute to their growth delay.

In the present study, all SS children commenced the adolescent growth spurt before 16 years of age, except for 8 cohort SS boys who remained prepubertal at this age. This group with extreme growth retardation, (defined as absence of the adolescent growth spurt and prepubertal sexual development [Tanner stage 1 or 2] at age 16 years) could represent the extreme of the normal growth spectrum in SS disease or a distinct subgroup with a different mechanism for their growth delay. Endocrine investigation of this subset of boys showed a

sub-optimal testosterone response to human chorionic gonadotrophin and an exaggerated gonadotrophin response to gonadotrophin hormone releasing hormone in 5/8 boys, consistent with impaired testicular function (Singhal *et al*, 1995). Therefore although most SS children had moderate delay in onset of puberty, potentially correctable mechanisms were identified in a subset of boys with extreme retardation of maturation.

The aetiology of retarded growth in <u>most</u> other patients with SS disease who have only moderate delay in puberty is probably multifactorial and cannot be explained by abnormal endocrine function. Increased metabolism secondary to high erythropoietic activity is a possible contributing factor but investigating the effect of increased erythropoiesis on growth and metabolism is limited by an inability to measure erythropoietic activity in a study group large enough to detect small differences in growth. Patients with chronic hypersplenism however provided a model of the detrimental effect of high erythropoietic activity. Splenectomy for chronic hypersplenism was associated with an increase in height but not weight velocity raising the possibility that reduction in erythropoietic stress ameliorates a specific nutrient deficiency thereby allowing an acceleration in linear growth. Although the validity of hypersplenism as a model of increased erythropoietic activity in SS children without hypersplenism is uncertain, this hypothesis may have implications for the growth abnormalities in non-hypersplenic SS children who also have increased erythropoiesis. Small numbers of hypersplenic children precluded investigation of gender differences in the effect of erythropoiesis on growth, but future investigation of the metabolic effect of splenectomy for hypersplenism could help elucidate mechanisms for growth failure.

The beneficial effect of high HbF levels on linear growth and the acceleration in growth following splenectomy for hypersplenism support the hypothesis that increased erythropoietic activity contributes to growth failure in SS children. The present study therefore investigated the metabolic consequences of erythropoiesis and in particular the effect on energy metabolism.

CHAPTER 3: NUTRITION, GROWTH AND ENERGY EXPENDITURE

NUTRITION AND GROWTH

Many acute dietary and other environmental factors such as a febrile illness reduce the rate of linear growth. Removing the impediment to growth usually produces a compensatory increase in growth rate or 'catch up' growth (Ashworth and Millward, 1986) that returns the child to his original genetically determined growth pattern. Repeated insults such as chronic malnutrition may reduce the ability to catch up in height and malnourished children compensate by delaying skeletal maturation and the onset of adolescent growth (Tanner 1962, Eveleth 1985). These mechanisms allow a longer period for prepubertal growth, the effect on final height depending on the severity of the nutritional deficiency and the degree of delay in ossification of epiphyses.

The pattern of growth in children with SS disease (reduced weight, height and skinfold thicknesses, delay in skeletal maturation, and retardation of puberty) is consistent with chronic childhood malnutrition. Delayed growth but a normal adult height suggests that energy and nitrogen balance is maintained over the entire growth period but SS children are in a state of adaptation and so may be less able to tolerate reduction in dietary protein or nitrogen. Poor healing of leg ulceration, a high susceptibility to infection and a high rate of metabolism may therefore reflect an inability of certain SS patients to cope with extra environmental stresses.

The First Growth Limiting Nutrient

An increase in body mass, or growth, requires appropriate amounts of all constituents of lean tissue, and energy for new tissue synthesis, deposition and remodelling. Any component least available relative to demands for lean tissue synthesis will limit the rate of growth and all other components in excess will be used inefficiently. Possible nutrients limiting growth in SS disease are unknown but a growth spurt, improvement in haematology, and fall in protein turnover following splenectomy for hypersplenism suggests that high metabolic requirements for erythropoiesis may compete with and limit growth in SS children.

The requirements for erythropoiesis and growth include energy, certain amino acids, iron, folic acid and other vitamins but which nutrients are insufficient relative to demand and therefore potentially growth limiting is not known.

The Evidence for Energy Deficiency

Energy requirements for growth are small relative to total energy consumption (15-30% at birth, falling to only 5% at one year of age (FAO/WHO/UNU, 1985) but in the face of higher requirements for erythropoiesis an energy deficiency may limit growth in SS disease. A theoretical energy saving of 22 $kJ.kg^{-1}.d^{-1}$ (sufficient energy for the growth rate of a one year old child; Spady *et al*, 1976) and growth spurt following splenectomy for hypersplenism is consistent with this hypothesis although supporting evidence for an energy deficiency in SS disease is scanty. Acceleration in height of growth retarded boys following nasogastric nutrient supplementation (Heyman *et al*, 1985) supports a nutritional deficiency in SS disease although the unphysiological method of feeding and poor design of this study make it impossible to elucidate which dietary factor is growth limiting. The failure of oral supplementation to promote growth suggests appetite suppression in patients and a normal energy intake in the face of a 20% increase in RMR is consistent with the concept of relative anorexia in SS disease. Whether the increase in RMR in SS disease leads to an energy deficiency and consequently adaptive mechanisms to conserve energy by reducing growth and physical activity (Touron 1990) is uncertain. The determinants of the increase in RMR in SS disease are unknown and may include both genotype differences in body composition and erythropoietic activity. Alternatively in the face of a specific growth limiting nutrient energy will be wasted metabolising nutrients that are in excess of requirements, and this mechanism could increase RMR and suppress appetite in SS patients (Kleiber 1945).

BACKGROUND TO METHODS

Aim

The aim of the present study was to investigate the determinants of the increase in RMR in SS disease in relation to the effects of body composition and erythropoietic activity. RMR was measured by indirect calorimetry and possible effects of a high RMR on growth and physical activity also investigated.

Principles of Indirect Calorimetry

Calorimetry is the measurement of heat either directly by physical methods or indirectly by quantitative measurement of the chemical by-products of metabolism. Indirect measurement of heat production uses the principles of the Law of Conservation of Energy (energy cannot be created or destroyed, only changed in form) and the Hess Law of Constant Heat Summation (the heat released by a chain of reactions is independent of the chemical pathways and dependent only on the end products). Indirect calorimetry depends on 2 assumptions:

1. That the end result of all the biochemical reactions which occur in the body amounts effectively to the combustion or synthesis of carbohydrate, fat and protein.

2. That for each of these substances, when it is oxidised in the body, there are fixed ratios between the quantities of oxygen consumed and carbon dioxide and heat produced.

Although these assumptions oversimplify the metabolic processes (for by example ignoring mineral metabolism and assuming a uniformity in the properties of all fat and protein despite their complex chemical structure), results of indirect and direct calorimetry are remarkably similar (McClean and Torbin, 1987).

Calculations

The amount of heat produced (M) is estimated from analysis of respiratory gases employing factors based on the heats of combustion and elementary composition of carbohydrate, fat, protein and urinary nitrogen substances:

Calculations can be reduced to :

$$M = aVO_2 + bVCO_2 + cN + dVCH_4 \text{ (kJ)}$$

Where VO_2 is oxygen consumption, VCO_2 is carbon dioxide, N urinary nitrogen and CH_4 methane (in ruminant animals) production. The values of the factors *a, b, c, d,* may vary slightly according to the nutrition, activity and growth pattern of the subject and a variety of formulae have been derived to calculate human energy expenditure. In the present study the formula of Weir (1949), assuming a non protein RQ, is used:

$$EE \text{ (KJ)} = 16.50 \, VO_2 + 4.63 \, VCO_2$$

In most equations the O_2 term contributes approximately 80%, the CO_2 term approximately 20% and the nitrogen term (which is very variable) less than 1% to the total energy expenditure.

The Ventilated Hood System

Oxygen consumption and CO_2 production for indirect calorimetry can be measured by closed and open circuit systems (McLean and Tobin, 1987). In the open circuit system used in the present study, air is drawn through a plastic hood (which is placed over the head and shoulders) by a pump (Rotameter Ltd) situated at the end of the system. The subject inspires from and expires into the air stream and the hood allows mixing of inspired and expired gases prior to drying with anhydrous calcium chloride, measurement of flow rate and gaseous analysis.

The flow rate (approximately 1 $L.kg^{-1}.min^{-1}$ of bodyweight) is adjusted to produce a change of less than 1% in the hood concentrations of oxygen and carbon dioxide since a higher inspired CO_2 concentration produces an increase in respiratory effort and energy expenditure. Measurements of temperature, pressure and humidity made at the same time as gas sampling are used to adjust the

observed flow rate to standard temperature and pressure for dry air (0°C, 760 mm Hg, and 0% humidity). The volume flow rates are corrected for water vapour, and the true O_2 consumption and CO_2 production estimated using the gas concentrations into and out of the hood and the corrected volume flow rates. The O_2 decrease or CO_2 increase is given by:

$$F_o f_{Go} - F_i f_{Gi}$$

where F is flow rate, f is fractional concentration, i is ingoing and o is outgoing, and G is any gas. Assuming conservation of N_2 in the chamber under steady state conditions:

$$F_i f_{N2i} = F_o f_{N2o}$$

with the concentration of N_2 being defined as:

$$F_{N2} = 1 - f_{O2} - f_{CO2}$$

The true flow rate for a gas is therefore given by

$$F_i f_{N2i} [(f_{Go} / f_{N2o}) - (f_{Gi} / f_{N2i})]$$

Adjusting the flow rate allows the ventilated hood system to be used for different size subjects and makes the system highly flexible. The system is also robust under field conditions particularly since the hood is well tolerated by children and negative pressure throughout the calorimeter eliminates leakage of expired air to the atmosphere. The main disadvantage of this method is that it requires accurate measurement of gas concentration differences of less than 1% between outlet and inlet air although using well calibrated, paramagnetic oxygen analysers with a range extending from 0-21% O_2, it is possible to calculate heat production with 1.2% accuracy (McLean and Tobin, 1987).

Assessment of Body Composition

Comparisons of RMR between individuals or groups should be adjusted for differences in mass of metabolically active tissue. Body weight is the simplest measure of metabolic mass but consists of tissues of different metabolic activity

with fat being relatively metabolically inert. The fat free or lean body mass (LBM) is the major determinant of RMR (Cunningham 1980) and is therefore indirectly measured in most studies of energy expenditure.

Since the earliest research (Behnke), most measurements of human body composition divided the body into 2 compartments, the fat and fat-free mass. In this model, the chemical composition of fat and fat free tissue, determined by cadaver analysis, is assumed to be known and relatively constant. A density of 1.1 g/cc at 37°C, a water content of 72-74%, and a potassium content of 60-70 mmol/kg in men and 50-60 mmol/kg in women is used for the fat free-body and fat is assumed to be anhydrous, potassium free and with a density of 0.900 g/cc at 37°C (Lukaski 1987). Indirect techniques for determining body composition utilise these differences in the fat and fat free body.

The simplest methods of determining fat mass use calipers to measure the skinfold thickness or subcutaneous adipose tissue and have the advantages of low cost and non-invasiveness. Equations can then be used to convert skinfold thickness to total body fat mass as the latter is closely correlated with subcutaneous fat mass. However few such equations have been validated in populations other than those in which they have been derived and most are likely to be inaccurate in SS patients who have abnormal body habitus and fat distribution. Newer techniques such as photon absorptiometry (which involves scanning the body with gamma rays of differing intensity from a radioactive source), and measurement of total body potassium (and hence fat free mass) using radioactive potassium-40 are contraindicated in children. The high degree of technical cooperation required for underwater weighing (using Archimedes principle and differences in the density of fat and fat free tissue) also makes densitometry unsuitable for children.

Total Body Water

For the purposes of the present study the two compartment model was used to determine lean body and fat mass. The LBM was derived from measurement of

total body water (TBW) a method which assumes that fat is anhydrous, and that water is a relatively fixed fraction of the fat free mass (73.2%).

Total body water is measured using an isotope dilution technique based on the relationship $C_1V_1 = C_2V_2$ where C_1V_1 is the amount of tracer isotope given, and C_2 is the concentration of the isotope in body fluids once equilibrium is reached and V_2 is the volume of TBW (after correcting for urinary loss of isotope). This method assumes that the isotope tracer has the same distribution volume as water, is exchanged by the body in a similar manner to water and is non toxic. Deuterium oxide is a suitable isotope that fulfils these criteria and can be used in combination with isotope ratio mass spectrometry (Halliday and Miller,1977) to determine TBW to an accuracy of $\pm$ 0.5%. However the assumption that the water concentration of lean tissue is similar in SS and AA patients and constant with age (although children have a higher water content) may be a source of error since the composition of LBM may change in disease states and with growth. Furthermore as the adolescent growth spurt is accompanied by increases in muscle, lean and fat mass, and is delayed in SS disease, ideally genotype comparisons of body composition should be confined to either prepubertal or post-pubertal subjects.

Muscle Mass

Comparative studies in adolescents highlight the problems of using a simple 2 compartment model of body composition. Body composition changes from childhood through adolescence to adult life but adolescents may be at different stages of physical maturity despite similar secondary sexual development. Measuring body composition using LBM alone may be insufficient since the LBM consist of tissues with different rates of metabolic activity, visceral tissues having higher rates than muscle. Differences in the proportion of visceral to somatic tissue will affect RMR and assessment of body composition in present study therefore included a measure of muscle mass estimated from the 24 hour excretion of creatinine.

Creatinine is formed by the non-enzymatic hydrolysis of free creatine liberated during the dephosphorylation of creatine phosphate. As creatine is 98% located in skeletal muscle the 24 hour urinary creatinine excretion can be used to estimate total body muscle mass although a large intra-individual variability in effect of diet and renal handling suggest that daily urinary creatinine excretion is to some degree independent of body composition (Lukaski 1987). Nevertheless, provided an accurately timed 24 collection is obtained, urinary creatinine measurement allows comparison of muscle mass between groups matched for age, sex and physical maturity and estimation of muscle mass to an accuracy of 3 kg (Lukaski 1987) .

RESTING METABOLIC RATE IN SS DISEASE

Studies on RMR in SS disease, limited to African reports of 8 adolescent males (Odonkor *et al*, 1982a) and 3 females (Apatu *et al*, 1988), a Jamaican report of 6 adult males (Badaloo *et al*, 1989) and an American report of 9 prepubertal children concluded that RMR was increased in SS disease. The validity of these conclusions, however, is limited by small study size, ascertainment biases, lack of reference data for body mass, and lack of appropriate control observations. In the present study RMR was determined in a representative sample of adolescents with SS disease and compared with AA controls matched for age, sex and pubertal stage. The effect of genotype differences in lean body mass on RMR were also investigated.

Subjects and Methods

Resting metabolic rate was determined in all available post-pubertal (Tanner Stage 4 or 5) SS adolescents from the cohort study and their AA control most closely matched for pubertal stage. These were 20 pairs (8 male, 12 female), aged 15 to 17.4 years who were clinically well at the time of the study and had not been transfused in the preceding 4 months.

All subjects fasted overnight and were brought to the metabolic laboratory in a car. Measurements were conducted between 10:00 and 12 noon in an air-conditioned room (22 - 25°C). After 30 minutes lying supine on a couch, a hood was placed over the head and shoulders and 10 minutes allowed for equilibrium of expired gases. Two consecutive 5 minute samples of expired air were then collected in Douglas bags, the flow rate in the hood being adjusted between 30-40 L/minute in order to maintain the CO_2 content of the expired air between 0.5-0.7%. Air samples were dried before entering the flow meter and again before measurement of the O_2 (Paramagnetic oxygen analyser, Servomex Ltd.) and CO_2 content (Infrared CO_2 analyser, Analytical Development Company Ltd). The

analysers were calibrated using 99.99% nitrogen for zero, and fresh air and 0.82% CO_2 for the oxygen and CO_2 span calibrations respectively. The metabolic rate was calculated using the method of Weir (1949) and the mean of two RMR measurements expressed as $MJ.kg^{-1}.d^{-1}$ were used for analysis. RMR measurements were repeated in all SS subjects 1-5 months later to assess the stability and reproducibility of values.

Anthropometry was measured as previously described and LBM derived in fasting subjects from the measurement of total body water using deuterium oxide dilution (Halliday and Miller, 1977). After the bladder had been emptied and urine collected to determine background enrichment, subjects were given a loading dose (2 ml/kg) of a 10% deuterium oxide solution. Urine samples were collected at 2,3,4, and 5 hours after loading and the enrichment of deuterium determined using isotope ratio mass spectrometry at the Dunn Nutrition Unit, Cambridge. Lean body mass was calculated from TBW assuming 73.0% hydration of the lean body. Haematologic indices and HbF were measured as previously described.

Statistical Analysis

Differences between patients and controls in RMR, haematology and anthropometry were assessed with the Wilcoxon's matched-pairs signed rank sum test. The contribution of covariates (differences in LBM, height, Hb concentration, HbF level and absolute reticulocyte count) to the variation in RMR differences between matched pairs was assessed by multiple regression, with the aim of identifying the most economical model. Previously RMR has been expressed per kg body weight (Badaloo *et al*, 1989) but as the relationship of RMR and LBM has a positive intercept on the y axis this method underestimates metabolic size in those with small LBM and overestimates in those with a larger LBM (Ravussin and Bogardus, 1989). Multiple regression analysis is a more appropriate way of analysing these data and was used to compare between group differences in the slopes and intercepts of the regression lines between RMR and LBM.

Repeatability of RMR measurements was assessed using the method suggested by Bland and Altman (1986).

Results

Patients with SS disease were lighter, had smaller arm circumference and lower metabolic mass and body mass index than AA controls (Table 13). Median total Hb levels in patients were lower and HbF levels higher than in AA controls. There were no statistically significant genotype differences in height, sum of skinfolds, or estimated lean body mass although all tended to be lower in SS subjects. The RMR was elevated in SS subjects whether expressed as $MJ.d^{-1}$, $MJ.kg^{-1}.d^{-1}$, or in relation to weight$^{0.75}$, or LBM (Table 14). The mean RMR in SS subjects was 9.5% (0.54 $MJ.d^{-1}$) or 18.9% (0.025 $MJ.LBM^{-1}.d^{-1}$) greater than in AA controls. The median RMR was also 15.1% (range: -4.1 to 34.6%) higher than values predicted from the Schofield (1985) equations. The respiratory quotient (RQ) was similar in the two genotypes. Paired estimates of RMR calculated from the two 5 minute consecutive gas collections gave a coefficient of repeatability of 0.01 $MJ.kg^{-1}.d^{-1}$ in AA and SS subjects. For RQ measurements the coefficients of repeatability were 0.11 and 0.07 in AA and SS subjects respectively. The coefficient of repeatability of RMR measurements in SS subjects 6 to 20 weeks later was 0.015 $MJ.kg^{-1}.d^{-1}$.

The RMR correlated with lean body mass in SS (r=0.71, p<0.001) and AA (r=0.82, p<0.001) subjects (Figure 4). Multiple regression analysis indicated that differences in LBM accounted for 21% of the variation in RMR differences between genotypes and after allowing for this, the RMR in SS subjects was on average 0.74 $MJ.d^{-1}$ (95% CI: 0.25 to 1.23) greater than in AA controls. Adding sex to the model revealed a striking difference, females showing a mean genotype difference of 0.31 $MJ.d^{-1}$ (-0.20 to 0.82) and males of 1.36 $MJ.d^{-1}$ (0.76 to 1.96). Genotype differences in Hb and HbF levels and reticulocytes made no additional contribution to the model.

Table 13
Comparison of some Characteristics of SS Adolescents and AA Controls

Variable	AA controls (n=20) Median	Range	SS disease (n=20) Median	Range	Wilcoxon test p
Age (years)	16.2	15.0-17.4	16.2	15.0-17.3	0.42
Weight (kg)	55.5	42.4-86.6	48.0	39.3-59.7	0.03
Height (cm)	168.9	151.4-181.9	165.3	154.5-180.2	0.18
Metabolic mass (weight$^{0.75}$)	20.3	16.6-28.4	18.2	15.7-21.5	0.025
Body mass index (kg/m^2)	18.9	15.3-29.5	18.1	13.8-22.5	0.044
Arm circumference (cm)	24.6	19.9-33.7	23.5	19.8-25.7	0.028
Sum of skinfolds (mm)	34.2	17.0-122.1	33.0	16.3-49.2	0.10
Lean body mass*(n=19)	45.5	33.8-67.1	40.1	32.2-56.2	0.084
Total haemoglobin (g/L)	134.0	93-162	82.0	65-112	<0.001
Fetal haemoglobin (%)(n=19)	0.2	0.1-0.7	6.1	0.4-13.4	<0.001

*Lean body mass in kg estimated from total body water measurements

Table 14
Resting Metabolic Rate in SS Adolescents and in AA Controls

Mode of expression	AA controls (n=20) Median	Range	SS subjects (n=20) Median	Range	p value
MJ.d^{-1}	6.00	4.92-7.76	6.31	5.02-8.65	0.015
MJ.kg^{-1}.d^{-1}	0.11	0.09-0.13	0.13	0.10-0.18	<0.001
MJ.(weight$^{0.75}$)$^{-1}$.d^{-1}	0.30	0.25-0.35	0.35	0.28-0.46	<0.001
MJ.LBM^{-1}.d^{-1}*(n=19)	0.14	0.11-0.15	0.16	0.13-0.19	0.002
RQ	0.82	0.72-0.97	0.83	0.72-0.91	0.72

*LBM = Lean body mass (in kg) estimated from total body water measurements

Figure 4: Relationship Between Resting Metabolic Rate and Lean Body Mass in Subjects with SS Disease (●) and in AA Controls (o).
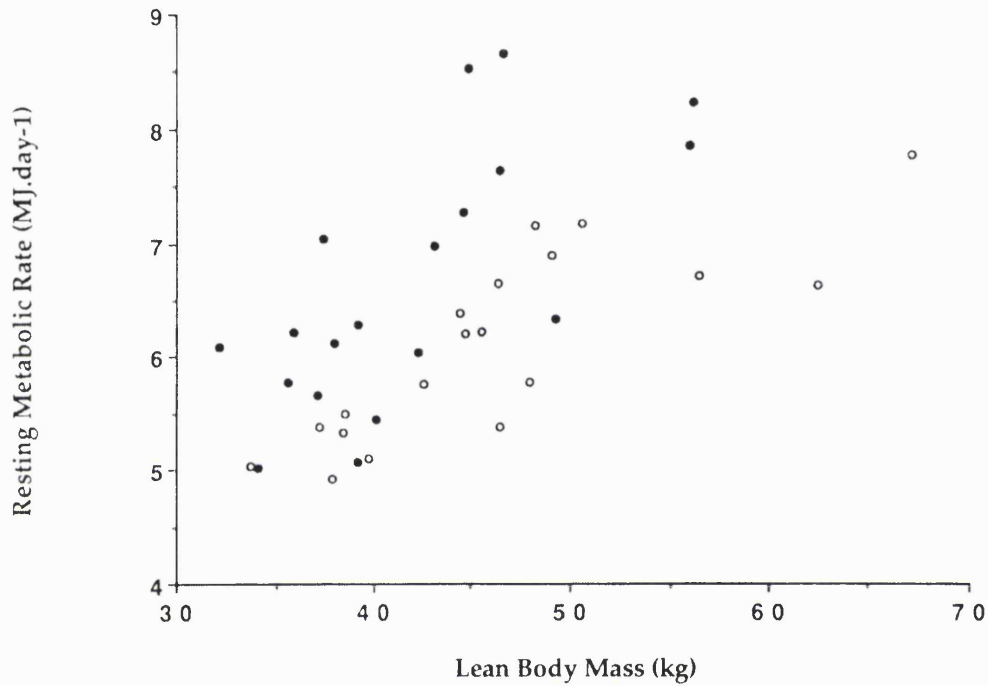
Discussion

The resting metabolic rate represents the minimum energy expended by a awake, fasted individual, resting supine in a thermoneutral room and appears to be highly reproducible in normal subjects (Soares and Shetty, 1987). The observation that estimates of RMR repeated in SS subjects after 6 to 20 weeks were also highly reproducible argues against this elevation being the spurious result of acute complications and suggests that there is a real and sustained elevation of RMR in SS disease.

The mechanism of the increased RMR in SS disease is uncertain. The lean body mass is a major determinant of the resting energy expenditure in normal individuals (Cunningham 1980) and correction for LBM increased the genotype difference in RMR from 0.54 to 0.74 MJ.d^{-1}. It is theoretically possible that a relative increase in visceral to somatic mass in SS disease could contribute to a higher RMR and such an explanation has not been excluded by the present study.

The thyroid hormones thyroxine and tri-iodothyronine may increase the RMR, but this mechanism is discounted by the lower tri-iodothyronine levels observed in SS disease (Parshad *et al*, 1989). Cardiac output is elevated in SS disease and the increased cardiac work could contribute to the increased RMR. However, the haemoglobin level is a determinant of the cardiac output and the lack of a relationship between haemoglobin and RMR is not consistent with this mechanism. Protein turnover has been estimated to account for 15 to 20% of basal energy expenditure in normal subjects (Waterlow 1990) and it is of interest that the 80% increase in protein turnover observed in SS disease (Badaloo *et al*, 1989) approximates to the 19% elevation in RMR.

Summary

The resting metabolic rate (measured by indirect calorimetry) was 19% higher in 20 patients with SS disease than in 20 age and sex matched AA controls. The difference was not accounted for by differences in lean body mass (measured by total body water).

ASSESSMENT OF ERYTHROPOIETIC ACTIVITY

Investigating the possibility of high erythropoietic activity increasing RMR in SS disease required a simple method of measuring erythropoiesis in a large number of patients. In the past, ferrokinetic studies measured the turnover of plasma iron but as these techniques are complex, expensive and use radioisotopes, the present study assessed the use of the serum transferrin receptor (TfRs) concentration as an assay for erythropoietic activity.

Transferrin receptors control the uptake of circulating transferrin and iron into cells (Huebers and Finch, 1987). High concentrations occur on the membrane of cells with high iron requirements such as red cell precursors and other rapidly dividing tissues (Kozlowski *et al*, 1988). As the cells mature, TfRs are cleaved from the cell surface and are detectable free in the plasma (Kohgo *et al*, 1986). Serum TfR levels, measured by immunoassay, correlate with ferrokinetic measures of erythropoiesis (Heubers *et al*, 1990), are elevated in iron deficiency anaemia, sickle cell disease and thalassaemia, and reduced in aplastic anaemia (Kohgo *et al*, 1986; 1987; Flowers *et al*, 1989; Huebers *et al*, 1990). Serum TfR levels could therefore provide a useful method of estimating erythropoietic activity in a large study population and levels were measured in the present study in normal children and in patients with SS, or SC disease, to explore their possible clinical value as an index of erythropoietic expansion. The determinants of serum TfR levels and the effect on serum levels of a variety of clinical complications of SS disease were also investigated.

Subjects and Methods

The patients were drawn from the cohort study and other 'non cohort' patients acquired predominantly by symptomatic referral to the sickle cell clinic. The distribution of serum TfR levels was measured in 270 cohort children (41 AA, 182 SS, 47 SC) representing all available sera stored at -70°C on their eighth birthday.

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The relationship of serum TfR values to steady-state total Hb, HbF, MCV, absolute and percentage reticulocyte counts measured on a simultaneous blood sample was assessed by correlation coefficient analysis. The relationship between serum TfR levels and growth was assessed in 28 cohort children with SS disease (14 males, 14 females) with the greatest height increment between 3-9 years compared with 28 children (14 males, 14 females) with the lowest height increment during this period. Longitudinal trends in this group were also assessed in all stored sera in three age bands (1-3, 4-6, and 7-9 years).

The influence of painful crisis on serum TfR was assessed by comparing levels in 30 SS patients (11 males, 19 females: median age 17.6 years, range 2.3-43.9 years) during bone pain with levels in the closest steady-state stored serum sample in the same patient. The effect of gross erythropoietic expansion on serum TfR concentration was assessed in 8 SS patients with hypersplenism before and after splenectomy. The effect of erythroid maturation arrest on serum TfR levels was assessed by longitudinal observations in 7 patients (3 SS, 4 SC) in aplastic crisis, as previously defined (Goldstein *et al*, 1987) and during the recovery phase. Iron deficiency, defined as either a serum transferrin saturation below 15% or a serum ferritin level <12 ug/L, occurred in 34 SS patients.

Serum TfR levels were measured by a sandwich, enzyme linked, immunoabsorbant assay in the laboratories of Dr James Cook on frozen serum samples shipped in dry ice from Jamaica. All determinations were performed in triplicate after dilution of all sera to 1:5000 because of the elevated serum TfR levels in patients with SS disease. Since some of the sera were stored at $-70^{\circ}C$ for up to 10 years, sample age was plotted against serum TfR level and failed to show any relationship suggestive of deterioration of samples.

Results

Serum Transferrin Receptor Levels and Genotype

Serum TfR levels at the age of 8 years were markedly elevated in SC disease compared to AA controls (p<0.001) and in SS disease compared to SC disease (p<0.001) (Table 15; Fig 5). Values were normally distributed and the means were compared by the unpaired t test using separate variance estimates when necessary.

Serum TfR Levels and Sex

Serum TfR levels were significantly higher in females in AA controls (t= –2.24, p=0.03) and in males in SS (t=2.41, p=0.02) and SC disease (t=2.03, p=0.048). There was no significant sex difference in ferritin levels in SS disease (p=0.86) or in AA controls (p=0.41). Ferritin values were not available in SC disease.

Serum TfR Levels and Haematology

Serum TfR level correlated negatively with HbF in both sexes and with Hb and MCV in males only (Table 16) but not with reticulocytes (proportional or absolute counts). Serum TfR levels did not differ between patients with or without iron deficiency either in males (mean, SD: 39.6, 14.8 vs 40.6, 12.2 mg/L; p=0.74) or females (37.6, 10.4 vs 35.6, 12.6 mg/L; p=0.63).

Serum TfR Levels and Age

The effect of age was assessed in the 56 SS cohort patients in the growth subgroup in whom, contrary to the whole population, serum TfR values were highly skewed and required transformation ($\log_e$ serum TfR levels). Serum TfR levels were available in all three age bands (1-3, 4-6, and 7-9 years) in 34 subjects, in whom levels were significantly higher in the oldest compared to the youngest group (paired t-test, p=0.035) but not compared to the middle group. The age related change in serum TfR levels within individuals correlated with a change in reticulocyte count (Spearman rank correlation: r=0.38, n=32, p=0.017) and with a fall in HbF levels (r= –0.51, n=26, p=0.004), but not with changes in other haematological indices. Thus subjects with greater rises in serum TfR tended to manifest larger increases in reticulocyte count and smaller falls in HbF.

Table 15

Serum Transferrin Receptor levels in 8 year old Children with Sickle Cell Disease and in AA Controls.

| | AA Controls | | | SS Disease | | | SC Disease | | |
	n	mean (SD) [range]		n	mean (SD) [range]		n	mean (SD) [range]	
Boys	16	5.9 (1.7) [1.9 - 9.2]		100	40.4 (12.8) [12.4 - 83.9)]		25	26.1 (10.1) [2.8 - 45.6]	
Girls	25	7.2 (1.8) [2.9 - 12.4]		82	35.9 (12.3) [15.9 - 75.0]		22	20.5 (8.5) [4.9 - 37.8]	
Total	41	6.7 (1.9) [1.9 - 12.4]		182	38.3 (12.7) [12.4 - 83.9]		47	23.4 (9.7) [2.8 - 45.6]	

All genotype differences were highly significant (p<0.001)

Serum transferrin receptor levels in mg/L

Table 16

Correlation between Serum TfR Levels and Haematological Indices in SS Disease

		Log_{10} (HbF)	Hb	MCV	Reticulocytes (%)	Absolute
Boys						
	r	-0.32	-0.40	-0.31	0.15	0.12
	n	58	66	66	66	66
	p	0.02	0.001	0.01	0.23	0.33
Girls						
	r	-0.38	-0.27	-0.13	0.25	0.23
	n	51	52	51	51	51
	p	0.006	0.055	0.34	0.08	0.11

Figure 5: Serum Transferrin Receptor Levels according to Genotype
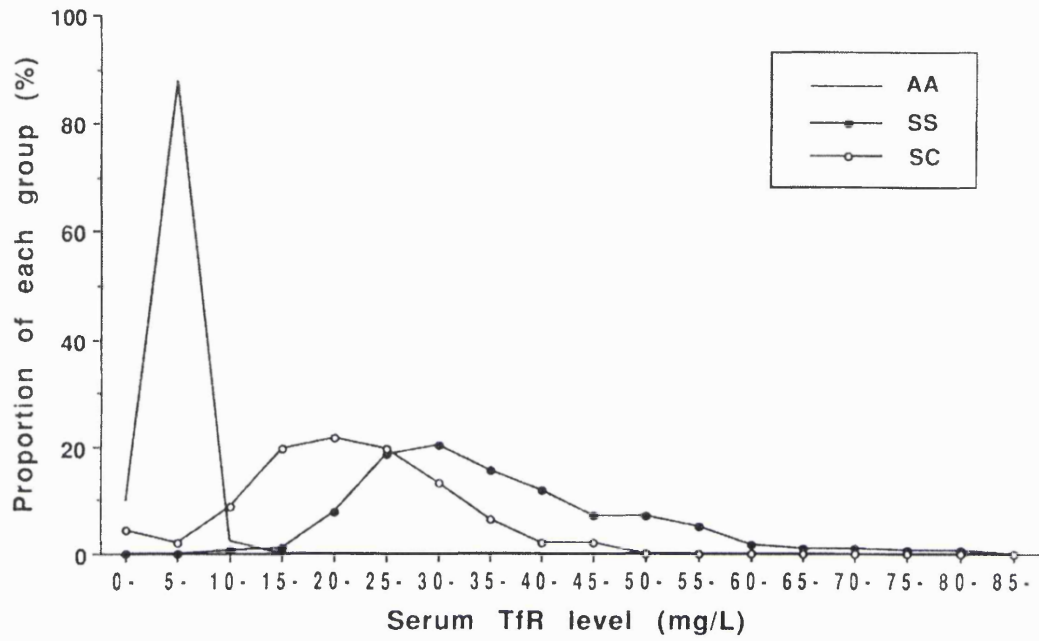
Figure 6:   Serum Transferrin Receptor Levels before and after

Splenectomy
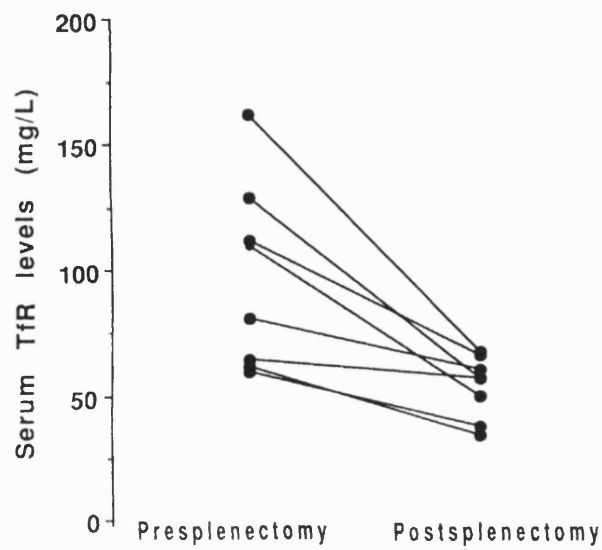
Serum TfR Levels and Height Velocity

In the growth subgroup of 34 subjects with serum TfR levels measured at each age, values at 7-9 years were compared in 18 and 16 patients with the greatest and lowest height velocities between 3 and 9 years. Serum TfR levels did not differ between patients with the greatest (median, [range]: 56.5 mg/L, [35.5 - 90.9]) or the least growth (58.3 mg/L, [34.5 - 114.4]; the t-test on the $\log_e$ transformed data gave p=0.58).

Variability in Serum TfR Levels

Analysis of between– and within–patient variation was confined to 34 SS patients with multiple assays (n=109) in subjects aged 4 years and above in whom no age related variation was demonstrable. In these subjects, intra-individual differences accounted for 47%, and inter-individual differences 53% of the variability in serially measured ($\log_e$ transformed) serum TfR values compared to corresponding figures for $\log_e$ reticulocyte counts of 86% and 14%. Serum TfR levels in comparison to reticulocyte counts thus tended to be characteristic for individual patients.

Serum TfR Levels and Clinical Complications

Serum TfR levels in 63 SS patients aged 8 years with a variety of inflammatory complications (25 with upper respiratory tract infections, 15 with painful crises, 7 with fever, 5 with skin infections, and 11 with other inflammatory events or infections) did not differ from levels in 119 SS patients of the same age in the steady-state (mean, SD; 37.7, 13.5 mg/L vs 38.7, 12.4 mg/L; p=0.61).

During painful crises serum TfR levels in 30 patients (median, [range]; 48.3 mg/L, [20.8 - 125.8]) did not differ from steady-state values in the same patients (44.8 mg/L, [18.0 - 88.8]) using the Wilcoxon matched pairs test (p=0.78).

Serum TfR levels were markedly elevated in 8 patients with chronic hypersplenism, values in the 7 patients aged 7-9 years (median [range]; 110.0 mg/L, [59.7 -162.6]) tending to be higher than in 34 steady-state cohort SS children of comparable age (57.1, [34.3 - 114.2]) (Mann-Whitney U-test gave

p=0.0069). Following splenectomy (Figure 6), serum TfR fell in all patients (Wilcoxon matched pairs test comparing pre- and post-splenectomy levels, p=0.028). Post-splenectomy levels in the 7 patients aged 7-9 years (57.0 mg/L, [34.1 - 67.4]) were similar to those in the 34 SS children in the steady-state.

Serum TfR levels during the early recovery phase of an aplastic crisis (6-13 days from presentation) (median [range], 46.1 mg/L, [19.3 - 66.8]) were greater than at 25-40 days (26.7 mg/L, [14.2 - 44.1], p=0.028) as were percentage reticulocyte counts (10.0, [2.0 - 24.0] % at 6-13 days and 4.0, [2.0 - 14.0] % at 25-40 days, p=0.035).

Discussion

Serum transferrin receptor levels are markedly elevated in patients with SS disease (Flowers *et al*, 1989) consistent with the increases observed in other haemolytic anaemias (Kohgo *et al*, 1986, 1987; Huebers *et al*, 1990). Levels in the present study were lower in patients with SC disease who have a moderate increase in haemolytic rate and varied widely between SS subjects, consistent with the variability in the haemolytic rates recognised in this condition (Bensinger and Gillette, 1974). Both observations support the hypothesis that serum TfR levels may indicate the degree of erythropoietic expansion. The negative correlation between HbF and serum TfR levels further supports this hypothesis since high levels of HbF inhibit sickling and haemolysis and might be expected to be associated with lesser degrees of erythropoietic activity.

The lack of a significant relationship between serum TfR levels and reticulocyte counts was not unexpected. A relationship has been reported in autoimmune haemolytic anaemia (Kohgo *et al*, 1987), but reticulocyte counts in SS disease are very variable and correlate only poorly with HbF levels (Maude *et al*, 1987) or other indices of haemolysis such as red cell survival (Serjeant *et al*, 1969). The density of surface transferrin receptors is high in immature erythroblasts and falls as the cells mature through the reticulocyte stage (Heubers

and Finch, 1987; Parmley *et al*, 1983). Furthermore the response to haemolytic stress includes not only an increase in production of erythrocyte precursors, but also a shift of precursors normally retained within the bone marrow. The overall effect of these factors is difficult to quantitate but would make unlikely a simple relationship between reticulocyte count and serum TfR level.

Iron deficiency in otherwise normal subjects increases the serum TfR level three to four fold (Kohgo *et al*, 1986, 1987; Flowers *et al*, 1989; Huebers *et al*, 1990). No increase was demonstrable in SS disease in the present study possibly because any effect of iron deficiency was obscured by the greater increase caused by the disease itself. Longitudinal studies within SS patients during iron deficient and iron replete phases may provide a more sensitive indicator of the effect of iron deficiency on serum TfR levels in SS disease.

Serum TfR levels increased with age in children with SS disease, suggesting an age related increase in erythropoietic activity. Greater erythropoietic activity should attend the rise in HbS levels and sickling consequent on the fall in HbF levels, so the negative correlation between the change in serum TfR and fall in HbF level was unexpected. This association is difficult to explain but stimulation of erythropoiesis may increase HbF production in animals (DeSimone *et al*, 1981) and this mechanism may also occur in SS patients.

Levels of serum TfR in complications of SS disease are consistent with its role as an indicator of bone marrow activity. The lack of a change in serum TfR levels during painful crises was not unexpected and was consistent with observations that the haemolytic rate is unchanged (Diggs 1956). High serum TfR levels in the recovery phase of aplastic crisis reflected the intense erythropoietic activity during recovery and levels paralleled changes in reticulocyte counts. In hypersplenism, where a marked erythropoietic expansion is largely relieved by splenectomy, high serum TfR levels fell to values in the normal range for SS disease following splenectomy reflecting the expected change in erythropoietic activity. Serial measurement of serum TfR levels may therefore help to distinguish

chronic hypersplenism from simple splenomegaly and identify children that could benefit from splenectomy. Serum TfR levels show less intra-individual variability than reticulocyte counts, and measurement of serum TfR may therefore be a reliable indicator of bone marrow activity.

Summary

Serum TfR levels were significantly elevated in SS compared to SC disease and in SC disease compared to AA controls. Females had higher levels than males in AA controls but lower levels than males in SS and SC disease. In SS disease, serum TfR levels rose with age from 2 to 8 years, the increase correlating with the increase in reticulocyte count and fall in fetal haemoglobin levels. Serum TfR levels did not change with infection or the painful crisis, were markedly increased in hypersplenism and fell dramatically following splenectomy for hypersplenism. These observations are consistent with the hypothesis that the serum TfR level may be a useful indicator of the degree of erythropoietic expansion in SS disease.

RESTING METABOLIC RATE AND ERYTHROPOIESIS

A high red cell turnover in SS disease may increase resting energy expenditure and this hypothesis was investigated by measuring serum TfR concentration, haematological indices and RMR in prepubertal SS boys. The hypothesis that a higher RMR retards prepubertal growth in SS boys was also assessed. The study was confined to prepubertal SS boys to eliminate the variable effect of puberty on both RMR and serum TfR levels, and because the genotype increase in RMR is greater in boys than girls with SS disease.

Subjects and Methods

RMR in Prepubertal Boys

All boys with SS disease attending the sickle cell clinic and aged between 8-11 years at the study date were eligible. The lower limit of 8 years was chosen because of the co-operation required for accurate RMR measurement and the upper limit to exclude boys showing signs of puberty. All subjects had been crisis free for 2 weeks preceding RMR measurement and were asymptomatic at the time of the study.

Resting metabolic rate was measured between 10.00-12.00 am using techniques previously described. The mean RMR from 2 five minute samples of expired air was calculated and expressed as an absolute value in $MJ.d^{-1}$ and as a percentage increase in observed compared to RMR values predicted from the Schofield (1985) equations. The heart rate was determined over the last minute of each 5 minute RMR measurement by manually measuring the pulse and the mean heart rate calculated. Height, weight, sitting height and skinfold thicknesses were measured as previously described and a sample of blood obtained for determination of haematological indices and serum TfR levels. Growth was depicted as a standardised score relative to the growth of a Jamaican cohort SS children and as the increment in height between the ages of 3 and 9 years.

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Repeatability of RMR Measurement

Previous RMR measurements in our laboratory were confined to adolescents but as poor patient co-operation from younger children could affect reliability, a pilot study assessed the repeatability of RMR measurement in prepubertal SS children. Asymptomatic prepubertal SS children (7 boys and 6 girls), aged 5.9 - 17.0 years (median: 8.9) and consecutively attending the sickle cell clinic for a routine visit were studied. Subjects were brought to the metabolic laboratory in a car and RMR and RQ measured as previously described on 2 occasions 24 hours apart.

Results

Repeatability of RMR Measurement

The mean difference between the first RMR value (mean, SD: 0.20, 0.06 $MJ.kg^{-1}.d^{-1}$) and the second RMR value 24 hours later was 0.001 $MJ.kg^{-1}.d^{-1}$ (SD: 0.004; standard error: 0.002 $MJ.kg^{-1}.d^{-1}$) with 95% of differences falling within 0.01 $MJ.kg^{-1}.d^{-1}$ or 6% of the group mean. For RQ the mean difference between the 2 values was 0.003 (standard error: 0.01) with 95% of differences falling within 0.07.

RMR in Prepubertal Boys

Some characteristics of the prepubertal boys in this study are given in Table 17. Resting metabolic rate was elevated in prepubertal SS boys (Table 18) compared to values predicted from the Schofield (1985) equations (median, [range]: 19.2%, [-15.4 - 37.1]) and the increase above predicted values was similar to the increase observed in the earlier study of SS adolescents compared to AA controls (median [range]: 15.1% [-4.1 - 34.6]).

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Table 17

Some Characteristics of 24 Prepubertal SS boys

Variable	Mean (SD)		Median (range)	
Anthropometry				
Age (years)	9.8	(0.8)	9.9	(8.3-10.8)
Height (cm)	129.0	(5.9)	129.3	(118.4-140.7)
Weight (kg)	23.2	(2.4)	22.9	(18.7-28.1)
Height increment (cm/yr)[1]	-		5.8	(5.3-7.7)
Height (z score)[2]	-0.34	(1.0)	-0.37	(-2.0-1.8)
Arm circumference (cm)*	16.5	(1.2)	16.5	(14.5-19.0)
Sum of skinfolds (mm)*	16.2	(3.2)	15.8	(12.1-22.7)
Haematology				
Hb (g/L)	73.0	(7.0)	73.0	(58.0-87.0)
HbF (% Hb)*	-	-	3.6	(0.4-17.8)
Haematocrit (%)*	21.3	(2.2)	21.0	(18.0-27.0)
RBC (x10^{12})	2.48	(0.3)	2.53	(1.92-3.12)
MCHC (mmol Hb/L)*	34.3	(2.3)	35.0	(30.0-38.0)
MCV (fL)	85.7	(7.4)	85.0	(69.0-102.0)
MCH (pg/cell)	29.5	(2.6)	29.0	(24.0-35.0)
Reticulocytes (%RBC)*	-	-	13.0	(8.0-28.0)
Platelets (x10^9/L)	484	(113)	439	(285-735)
Iron (umol/L)**	-	-	9.8	(4.2-20.7)
TIBC (umol/L)**	41.7	(6.9)	41.4	(32.4-54.3)
Transferrin Saturation (%)**	22.7	(9.0)	21.0	(9.0-38.0)
Serum transferrin receptor (mg/L)*	59.4	(22.9)	54.4	(21.6-118.0)

n=24 except when * n=23 **n=22
TIBC = total iron binding capacity
[1]Increment in height between 3-8 years of age
[2]standardised score relative to the Jamaican Cohort
Means (SD) not given when variable not normally distributed

<table>
<tr><td colspan="5" align="center">**Table 18**

Resting Metabolic Rate in 24 Prepubertal SS boys</td></tr>
<tr><td>**Variable**</td><td>**Mean**</td><td>**(SD)**</td><td>**Median**</td><td>**(range)**</td></tr>
<tr><td>RMR $(MJ.d^{-1})$</td><td>5.09</td><td>(0.60)</td><td>5.09</td><td>(3.52-5.92)</td></tr>
<tr><td>RMR $(MJ.kg^{-1}.d^{-1})$</td><td>0.22</td><td>(0.025)</td><td>0.22</td><td>(0.18-0.26)</td></tr>
<tr><td>Predicted RMR $(MJ.kg^{-1}.d^{-1})$</td><td>0.19</td><td>(0.10)</td><td>0.19</td><td>(0.17-0.21)</td></tr>
<tr><td>% difference in RMR[1]</td><td>15.9</td><td>(13.3)</td><td>19.2</td><td>(-15.4-37.1)</td></tr>
<tr><td>RQ</td><td>0.81</td><td>(0.04)</td><td>0.81</td><td>(0.76-0.91)</td></tr>
<tr><td>Pulse rate (beats/min)*</td><td>82.6</td><td>(15.4)</td><td>84.0</td><td>(56.0-108.0)</td></tr>
</table>

n=24 except when * n=23; MJ= mega joules

[1]Percentage difference in observed RMR compared to RMR predicted from Schofield equations: $\left(\dfrac{Observed\ -predicted}{predicted} \right)$ X 100

Determinants of RMR

Absolute RMR $(MJ.d^{-1})$ was negatively correlated with age (r= -0.37, p=0.07) and positively correlated with weight (r=0.47, p=0.02) and standardised score for height (r=0.63, p=0.001). The percentage difference in observed compared to predicted RMR was negatively correlated with age (r= -0.58, p=0.003), standardised (z) score for height (r= -0.43, p=0.03) and total iron binding capacity (r= -0.45, p=0.03) (Table 19).

Multiple regression analysis was used to identify the independent correlates of absolute RMR amongst those significant in the crude analysis and the strongest relationship was for age (partial correlation r= -0.52, p=0.012) and weight (partial correlation r=0.58, p=0.004). After adjusting for age and weight, absolute RMR significantly correlated only with the haematocrit (partial correlation r= -0.44, p=0.047). Age showed the strongest relationship with the percentage increase in observed compared to predicted RMR (partial correlation r= -0.69, p=0.0003) and after adjusting for age, only haematocrit remained significantly correlated (partial correlation r= -0.48, p=0.04) (Table 19).

Table 19

Correlations of RMR with Haematology and Anthropometry in 24 Prepubertal SS boys

Variable	Absolute RMR				% difference in RMR[1]			
	(Unadjusted)		(adjusted) [2]		(Unadjusted)		(Adjusted)[3]	
	r	p	r	p	r	p	r	p
Age	-0.37	0.07	-	-	-0.58	0.003	-	-
Height	-0.23	0.31	0.28	0.25	-0.43	0.03	0.25	0.27
Weight	0.47	0.02	-	-	0.08	0.72	0.21	0.37
Height increment [4]	0.27	0.20	-0.17	0.44	-0.10	0.63	0.02	0.93
Height (z score)[5]	0.63	0.001	0.38	0.08	0.43	0.03	0.36	0.11
Arm circumference *	0.25	0.26	-0.06	0.80	0.02	0.92	0.12	0.61
Sum of skinfolds *	0.42	0.04	0.23	0.33	0.23	0.29	0.27	0.24
Haematocrit *	-0.29	0.17	-0.44	0.047	-0.27	0.20	-0.48	0.04
RBC	-0.02	0.93	-0.25	0.26	-0.04	0.85	-0.17	0.49
MCHC *	0.27	0.21	0.29	0.20	0.18	0.42	0.29	0.23
MCV	-0.07	0.76	0.08	0.71	-0.08	0.70	-0.00	0.99
MCH	-0.08	0.72	0.08	0.73	-0.09	0.68	-0.01	0.98
In (Reticulocytes)*	-0.34	0.12	-0.16	0.48	-0.23	0.29	-0.13	0.58
Platelets	-0.29	0.17	0.06	0.78	-0.25	0.24	-0.09	0.70
NBC *	-0.29	0.18	-0.41	0.07	-0.20	0.36	-0.43	0.05
In (Iron + 1) **	-0.18	0.43	0.01	0.98	-0.25	0.26	-0.10	0.70
TIBC **	-0.48	0.03	-0.27	0.25	-0.45	0.03	-0.36	0.11
Transferrin saturation**	-0.01	0.98	0.06	0.82	-0.09	0.68	0.07	0.77
Serum TfR*	-0.14	0.51	-0.20	0.39	-0.21	0.35	-0.11	0.64
Pulse*	-0.04	0.86	0.22	0.34	0.01	0.96	0.10	0.64
In (HbF+1)*	-0.03	0.90	-0.08	0.73	0.08	0.73	-0.14	0.57

n=24 except when *n=23, **n=22   NBC= Nucleated blood cells

[1] Percentage ($\frac{\text{Observed RMR -predicted RMR}}{\text{predicted RMR}}$) X 100 ;

[RMR predicted using Schofield equations]
[2] Partial correlation adjusted for weight and age;
[3] Partial correlation adjusted for age;
[4] Increment in height between age 3-8 years;
[5] Standardised score relative to the Jamaican Cohort

Growth depicted as a z score for height was not significantly correlated with the adjusted absolute RMR (partial correlation r=0.38, p=0.08), adjusted percentage increase in observed compared to predicted RMR (partial correlation r=0.36, p=0.11), or with the serum TfR concentration (r= -0.08, p=0.72). Similarly, the increment in height between age 3-8 years was not significantly correlated with adjusted absolute RMR, the percentage increase in observed compared to predicted RMR (Table 19) or with the serum TfR level (r= 0.09, p=0.68).

Discussion

Resting metabolic rate in prepubertal boys is 16% greater than predicted from the Schofield equations. This increase is similar to that in SS compared to AA adolescent boys and suggests that the increase in RMR in SS disease is not a result of genotype differences in metabolism or body composition occurring at puberty. Although body composition has not been assessed in the present study, genotype differences in anthropometry and body composition increase with age and are likely to be smaller in prepubertal than post-pubertal boys. The higher RMR in both pre- and post-pubertal boys is therefore consistent with the hypothesis that the increase in RMR in SS disease is independent of genotype differences in body composition and the 23% increase in RMR, expressed relative to fat free mass, of prepubertal SS compared to AA children (Gray *et al*, 1992) supports this observation.

High metabolic activity of a hyperexpanded bone marrow is proposed as a mechanism for the increase in RMR in SS disease. However, in the present study there were no significant relationships between RMR and the reticulocyte count or a quantitative measure of bone marrow erythropoietic activity, the serum TfR concentration. HbF and Hb also did not correlate with RMR suggesting that anaemia and a compensatory increase in cardiac output did not contribute to a high RMR and this hypothesis was supported by the lack of a correlation between heart

rate and RMR. A low Hb level in the presence of high marrow activity in hypersplenic patients and an expansion of plasma volume underestimating red cell mass (Steinberg *et al*, 1977) suggest that Hb concentration alone may not accurately reflect bone marrow activity in SS disease, which may explain the lack of any relationship between Hb and RMR.

After adjusting for known determinants such as age and weight, only haematocrit negatively correlated with both absolute RMR and the percentage increase in RMR above predicted levels. There was no correlation of RMR with any other index of erythropoiesis including the red blood cell count (RBC) suggesting that the relationship between haematocrit and RMR may be spurious. However, haematocrit may be a better indirect measure of red cell mass than Hb concentration or RBC and therefore these data could suggest that SS patients better able to maintain red cell mass in the face of increased haemolysis have a lower RMR. The mechanisms for this observation are uncertain but it is possible to speculate that a specific nutrient deficiency may prevent optimal erythropoietic activity and therefore increase energy costs associated with ineffective erythropoiesis or metabolism of nutrients excess to requirements (Kleiber 1945). Which nutrients may be insufficient relative to demand is not known but the negative correlation of RMR with haematocrit and lack of a similar relationship between RMR and indices of iron status or serum TfR suggest that nutrients required for synthesis of erythrocytes rather than haemoglobin are more likely to be deficient. The level of ineffective erythropoiesis in SS disease is unclear since endogenously labelled red cells have not been used to estimate red cell turnover in recent studies (Badaloo *et al*, 1989). However, the lack of correlation of reticulocyte count (a measure of effective erythropoietic activity) with high serum TfR concentrations (a measure of erythropoietic mass) suggest that ineffective erythropoiesis may occur in SS disease. Studies of red cell turnover using endogenously labelled red cells could therefore help to determine the degree of

ineffective erythropoiesis and by measuring the red cell mass also shed light on the relationship between RMR and haematocrit.

The lack of significant correlation between growth and RMR in prepubertal boys suggests that a high RMR is not an independent determinant of growth retardation in SS disease and the similar RMRs of post-pubertal SS boys and SS boys of the same age with extreme retardation of puberty (Singhal *et al*, 1995) is consistent with this observation. The increment in growth between age 3-8 years or z score for height of prepubertal SS boys also did not correlate with serum TfR levels suggesting that erythropoietic expansion alone does not impair growth. However, many complex environmental factors affect growth in a heterogeneous population such as children with SS disease and it was not surprising therefore that the present study did not find an association between growth and a single variable such as RMR. Furthermore a high RMR does not necessarily effect energy balance or imply an energy deficient state. Behavioural adaptation may conserve or increase the intake of energy and the individual variability in this adaptation is likely to obscure any simple relation of RMR and growth.

Summary

The determinants of RMR in prepubertal boys with SS disease were investigated and no relations were found with HbF, Hb or serum TfR concentrations. Both the absolute RMR $(MJ.d^{-1})$ and the percentage increase in RMR compared to values predicted from the Schofield equations were negatively correlated with the haematocrit only, but an explanation for this observation is unclear.

The increment in height from 3-8 years of age and height expressed as a standardised score relative to the Jamaican Cohort of prepubertal SS boys were not correlated with the absolute RMR, percentage increase in RMR (compared to values predicted from the Schofield equations) or to the level of serum transferrin receptor.

RESTING METABOLIC RATE AND BODY COMPOSITION

Resting metabolic rate for any individual depends on the mass of metabolically active tissue. The RMR of SS adolescents is approximately 20% greater than that of AA controls when expressed relative to lean body mass measured by total body water, but this 2 compartment model of body composition may fail to adjust for genotype differences in the composition of the lean body. Relative to weight, SS patients like malnourished children with a normal haemoglobin genotype may have more non-muscular, metabolically active, visceral tissue than AA controls. The greater proportion of visceral mass to muscle mass explains the higher protein turnover per kg of LBM of malnourished adults (Soares *et al*, 1991) and the possibility of a similar explanation for increased RMR in SS patients was therefore investigated.

Subjects and Methods

All post-pubertal SS adolescents in the cohort study (Tanner stage 4 or 5) without leg ulceration or other chronic disease at the time of the study were elligible. The AA boy from the cohort study most closely matched for age and pubertal stage was selected as a control for each SS patient to give a total study group of 16 pairs aged 16.1-20.1 years. No subject had been transfused in the preceding 4 months, and all were clinically well at the time of the study and for the preceding 2 weeks.

Anthropometry was measured as previously described and LBM derived in fasting subjects from the measurement of total body water by using deuterium oxide dilution. Muscle mass was estimated from the 24 urinary excretion of creatinine starting at 0800 am, with urine stored in hydrochloric acid and kept at $-70^{\circ}C$ until analysed. The creatinine concentration was determined using the modified Jaffe reaction (Heingard and Tickerstrom, 1973) and an automated, colourimetric analyser (Sigma Diagnostics technical manual, St Louis, USA 1991) and muscle mass estimated from these values assuming that 60 mg of creatinine

corresponded to 1 kg of muscle (Soares *et al*, 1991). The latter conversion factor included a correction for the contribution of extracellular fluid to muscle mass (Barac-Nieto *et al*, 1978).

RMR was measured by indirect calorimetry as previously described on subjects fasted overnight (12-14 hours) and bought to the metabolic laboratory in a car. Genotype differences in RMR were adjusted for genotype differences in body composition using repeated measures analysis of variance.

Results

As expected, SS patients were lighter and with less LBM than their AA controls (Table 20). The absolute muscle mass and muscle mass as a proportion of body weight was greater in AA compared to SS adolescents. Resting metabolic rate ($MJ.d^{-1}$) was lower in AA than SS subjects and the genotype difference increased from $-0.7 MJ.d^{-1}$ (95% CI: -1.2 to -0.2) to $-1.4 MJ.d^{-1}$ (95% CI: -2.1 to -0.7) after adjusting for the LBM. Adjusting for muscle mass did not alter the mean genotype difference in RMR but adjusting for muscle mass as a proportion of body weight or non-muscle mass reduced the genotype difference to $-0.5 MJ.d^{-1}$ (95% CI: -1.0 to 0.06) (Table 21).

Discussion

Patients with SS disease have an aesthenic body habitus, reduced arm circumferences and less subcutaneous fat (Ashcroft and Serjeant, 1972; McCormack *et al*,1976; Stevens *et al*, 1983) but genotype differences in body composition have not been assessed. In the present study, muscle mass measured by the 24 hour excretion of creatinine expressed relative to body weight was lower in SS (41%) than AA adolescents (46%) and was similar to values in undernourished labourers (43%) and underweight Indian adult males (43%) (Soares *et al*, 1991). In contrast, the same index in AA controls was similar to normal weight Indians (46%) (Soares *et al*, 1991).

Table 20

Comparison of some Characteristics of 16 SS Subjects with 16 AA Controls

	AA Mean	(SD)	SS Mean	(SD)	Significance p
Age (years)	18.3	(0.9)	18.0	(1.1)	0.007
Height (cm)	175.1	(4.8)	170.5	(4.0)	0.023
Weight (kg)	58.8	(4.3)	51.8	(4.5)	0.001
LBM (kg)	56.7	(4.3)	49.1	(3.3)	0.001
Fat mass (kg)	2.1	(1.7)	2.7	(3.4)	0.038
Muscle mass (kg)	27.0	(3.3)	21.2	(3.3)	<0.001
Muscle mass/weight	0.46	(0.1)	0.41	(0.1)	0.051
Non-muscle/muscle mass	1.1	(0.2)	1.4	(0.2)	0.07
muscle/non-muscle mass	0.9	(0.2)	0.7	(0.2)	0.07
SES	13.5	(4.2)	13.8	(3.2)	0.84
RMR (MJ.d^{-1})	6.3	(0.5)	7.0	(0.9)	0.02

SES= socioeconomic score (Alleyne *et al*, 1979)

LBM= Lean body mass

Table 21

Genotype Differences in RMR after Adjusting for Genotype Differences in Body Composition

	Mean genotype difference RMR (MJ.d^{-1})	(95% Confidence Interval)
Unadjusted	-0.7	(-1.2 to -0.2)
Adjusting for:		
Weight	-1.3	(-1.9 to -0.7)
LBM	-1.4	(-2.1 to -0.7)
Fat mass	-0.7	(-1.3 to -0.1)
Muscle mass	-0.7	(-1.5 to 0.1)
Muscle mass/weight	-0.5	(-1.0 to 0.06)
Non-muscle/ muscle mass	-0.5	(-1.0 to 0.06)

Although it was not possible to measure visceral mass directly, similar fat and non-muscle mass in SS and AA adolescents suggest that genotype differences in weight were due to differences in muscle and not visceral mass. Mechanisms for the selective reduction in muscle mass in SS patients are unclear but consistent with chronic malnutrition affecting muscle bulk (Jackson 1990) but sparing vital organs and the early genotype differences in arm circumference and lower body mass index of SS children (Stevens *et al*, 1983) support this hypothesis.

Four organs (brain, liver, heart and kidney) are estimated to account for 67% of basal metabolic rate in adults (Holliday 1971) and therefore higher visceral mass relative to total LBM (or weight) in chronically malnourished individuals may increase RMR per kg of LBM (or per kg body weight). In the present study, adjusting for genotype differences in muscle mass and muscle mass to non-muscle mass ratio reduced the genotype difference in RMR ($MJ.d^{-1}$) from 11% to 8% which then just failed to reach statistical significance. This observation suggests that a relatively higher visceral mass in SS patients explains some but probably not all of the increase in RMR in SS disease.

Summary

Lower weight in SS adolescents compared to AA controls appeared to be predominantly secondary to lower muscle mass in SS disease.

The higher RMR of SS adolescent boys compared to age and sex matched AA controls just failed to reach significance after adjusting for the greater non-muscle to muscle mass ratio in SS subjects. This observation suggests that a greater visceral to total body mass ratio in SS compared to AA subjects explains some of the increase in RMR (per kg body weight or LBM) in SS disease.

TOTAL DAILY ENERGY EXPENDITURE

As resting metabolic rate is the major determinant of total energy expenditure (Garrow 1985, FAO/WHO/UNU 1985), a small increase in RMR will markedly increase total daily energy expenditure (TDEE) and hence energy requirements. In the face of a 20% increase in RMR (Odonkor *et al*, 1982a; Badaloo *et al*, 1989) SS patients have the options of increasing calorie intake or economising on energy expenditure for physical activity. Voluntary calorie intake may not differ from that in AA controls (Phebus *et al*, 1988, Tangney *et al*, 1989) yet supplementation that bypasses appetite control promotes growth (Heyman *et al*, 1985), suggesting a suboptimal nutritional state. Patients may therefore economise on energy expenditure by reducing physical activity and this hypothesis was investigated by comparing RMR and TDEE in SS adolescents with AA controls matched for age, sex and pubertal development.

Energy Expenditure in Free Living Individuals

The two basic approaches to the study of energy metabolism involve measurement of energy intake or energy expenditure. Energy intake studies however assume a state of energy balance, suffer from under-reporting of intake data and are being replaced by measurement of energy expenditure (FAO/WHO/UNU, 1985). Energy expenditure studies on the other hand are complicated by the need to determine the energy cost of physical activity and although activity questionnaires, diary records or direct observation of physical activity can be used to rank children's physical activity level these methods suffer from poor subject cooperation, under-reporting and interference of spontaneous daily activity. Values for energy costs of activities (expressed as multiples of RMR), and the time spent in each activity can also be used to estimate total daily energy expenditure but this factorial approach is subject to observer bias and may affect the parameter being measured. Only the double labelled water and heart rate (HR) methods

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produce minimum interference with the subjects physical activity and provide a reasonably accurate estimate of TDEE.

Double Labelled Water Method

Recording the relative turnover rates of two stable isotopes in serial urine samples after ingestion of a weighed dose of double labelled water ($H^2_2O^{18}$) allows the measurement of energy expenditure over a period of 8-14 days. H^2 labels the body's water pool and O^{18} labels both the water and bicarbonate pools and therefore H^2 is lost as water only and O^{18} as both water and CO_2. Carbon dioxide production can be estimated from the difference in O^{18} and H^2 rate constants and energy expenditure calculated from the equations of indirect calorimetry. This method assumes a constant respiratory quotient and an estimate for the proportion of water that is fractionated during evaporation from epithelial surfaces.

The double labelled water method in combination with RMR measurement provides a good estimate of the energy cost of physical activity and is suitable for children since minimum subject co-operation is required. Disadvantages include the high cost of the isotope (approximately £250 per study), technical demands of mass spectrophotometric analysis of samples, and an inability to analyse intra-individual variations in physical activity patterns. Furthermore in the absence of Jamaican data it is not clear whether rates of water fractionation derived from studies in other countries are valid for Jamaican SS subjects.

Heart Rate Method

The association of oxygen consumption with heart rate was first recorded by Benedict in 1907 and is the basis of the HR method for estimating energy expenditure. Early attempts to use this association, based on average HR over the entire study period, gave imprecise results since the average 24 HR was close to resting HR (Dauncey and James, 1979). Mean HR values therefore fell on the part of the O_2 consumption (VO_2)/HR calibration curve where physiological stimuli such as posture, muscle activity and emotions could increase HR without a corresponding increase in energy expenditure.

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The development of lightweight ambulatory HR monitors overcame many early problems by measuring HR (and hence energy expenditure) minute by minute (Spurr et al, 1988). Using this method only the active and most accurate part of the O_2 consumption/HR calibration curve is used and energy expenditure for low exercise levels estimated from RMR. Accuracy is further increased by determining individual VO_2/HR calibration curves (Li et al, 1993) since the relationship between oxygen consumption and HR varies with the fitness of the subject.

The accuracy of the HR method depends on the physical activity profile of the subject and is likely to be low for sedentary subjects whose HR is frequently on the least active part of the VO_2/HR calibration curve where the relationship is least secure. Using only HRs above a certain 'Flex' value to calculate VO_2 may decrease the error although inaccuracies are likely to arise form the arbitrary estimation of Flex HR. Another potential problem is the poor association between the spontaneous activities of children and the activities used to establish the VO_2/HR relationship in the calibration procedure. Energy consumption at a particular HR depends on the muscles being used, the type of contraction (static vs dynamic), the state of cardiorespiratory training and environmental factors. It has therefore been suggested that a spectrum of activities be used for calibration (Li et al, 1993) although this requires a great deal of patient cooperation. An additional concern is the time lag between the return of energy expenditure and HR to resting levels after a period of activity, the latter being slower and therefore tending to overestimate energy expenditure in the recovery period (Spurr et al, 1988).

Despite its inherent problems, validation studies suggest that the HR method can estimate TDEE of small groups rather than that of individuals. The mean error, using whole body calorimetry as a standard, was 2.5% although the precision of individual values ranged from +20% to -15% (Spurr et al, 1988). Similarly, using double labelled water as a standard, the mean error was 0.7% although individual discrepancies ranged from +52% to -22% (Livingstone et al, 1992). The unique ability of the HR method to estimate intra-individual physical

activity patterns, low cost and generally good compliance in field studies therefore made this technique suitable for studies of TDEE in Jamaica.

Subjects and Methods

All available post-pubertal (Tanner stage 4 or 5) SS boys in the cohort study who were free of leg ulceration and of chronic disease other than SS disease were studied. The AA boy from the cohort study most closely matched for age and pubertal stage was selected as a control for each SS patient to give a total study group of 16 pairs aged 16.1-20.1 years. No subject had been transfused in the preceding 4 months, and all were clinically well at the time of the study and for the preceding 2 weeks. All subjects were unemployed or occasionally employed and none were working at the time of the study. Anthropometry, socioeconomic status and resting metabolic rates were measured by methods previously described and TDEE estimated by the HR method. Indirect calorimetry was used to establish the individual relationship between oxygen consumption and HR and each SS/AA pair was studied on the same day for all measurements.

Calibration of Oxygen Consumption against HR.

The relationship between oxygen consumption and HR in fasted individual subjects was determined in a calibration procedure (Spurr and Reina, 1990; Spicher *et al*, 1991) conducted in the metabolic laboratory following RMR measurements. Oxygen consumption was measured at two levels of low activity: sitting and standing quietly. Ten minutes were allowed for equilibration of expired gases at each activity after which 2 five minute samples of expired air were collected in Douglas bags. Oxygen consumption at high activity was determined in a continuous procedure using a treadmill as this provides a physiological and familiar exercise and allows the work load (speed and gradient) to be controlled (although the energy expended at any work load depends on the subject's technique, with the energy cost of jogging for example being greater than of walking at low speeds). Unlike cycle ergonometry, a treadmill also allows the net mechanical work

efficiency (the ratio of external work to net energy expenditure expressed as a percentage; Gaesser and Brooks, 1975), to be calculated and this was determined for all subjects at 3 and 5 km/hr as follows:

Energy Efficiency =

$$\frac{\text{Body mass (kg) X Constant of gravity } (9.81 \text{ m. sec}^{-2}) \text{ X Altitude gained (m)}}{\text{Energy expenditure (joules) at 3 km/hr (or 5 km/hr) - RMR}}$$

Four activity levels were used for the VO_2/HR regression line (walking at 1.5 km/hr with zero gradient, and at 1.5, 3 and 5 km/hr with a 10% gradient) and for each activity 5-7 minutes were allowed for equilibration of gases and a three minute sample of expired air collected. For all activity levels a calibrated electrocardiograph monitor was used to measure HR per minute over the first and last minutes of expired gas collection and the mean HR for each activity level calculated. Calibration procedures were conducted on the day prior to or following TDEE measurement.

HR Measurement in Free Living Conditions

Chest electrodes, attached to a belt worn around the chest, recorded the HR averaged over one minute. This HR information was transmitted to and stored in a Polar HR analyser (Sports tester PE3000, Polar Electro, Kempele, Finland). This analyser is normally worn as a digital wrist watch but because of local conditions and possible vulnerability of subjects wearing a watch, the receiver was detached from the wrist strap and concealed in a small box attached to the chest belt. The HR monitors did not restrict movement, could be worn in the shower and were well tolerated.

Twenty-four hour HR recordings were loaded on to a computer that calculated the percentage of time spent at each HR interval of 10 beats per minute. Heart rate recordings were started at 10:00-12:00 hr on 2 consecutive non-working weekdays and were used for analysis if 23 hours data were obtained on

each day. If there was loss of contact between the chest electrodes and skin the procedure was repeated on the following weekday (repeated for 6/64 measurements, 3 SS and 3 AA subjects).

Calculation of Total Daily Energy Expenditure

TDEE was estimated by recording the HR over 24 hours, on the assumption that energy expended for a given activity is proportional to the HR of the individual. Indirect calorimetry was used to establish the individual relationship between energy expended and HR and each SS/AA pair was studied on the same day for all measurements. The flex HR was first determined for each individual as the mean of HRs at resting activities (lying supine, sitting and standing quietly) and lowest exercise activity (walking at 1.5 km/hr on a zero gradient). The flex HR can also be calculated as the mean of the highest HR during rest and the lowest HR during exercise (Ceesay *et al*, 1989; Livingstone *et al*, 1992) but as postural effects often meant greater HRs during standing than exercise, the former definition was used in the present study.

A first order regression of VO_2 on HR was calculated for high intensity (treadmill) exercises and VO_2 for time spent above flex HR determined using this relationship. For time spent below flex HR (including sleeping time) the mean VO_2 of resting activities (Livingstone *et al*, 1992) was used. The sum of VO_2 for time above and below flex HR was used to calculate TDEE employing 20.48 kJ/L as the calorific equivalent of VO_2 at a respiratory quotient of 0.88 (Spurr and Reina, 1990). The mean (SD) TDEE over 2 days was calculated and TDEE/RMR (an index of physical activity level, PAL) determined for each subject.

Differences between SS and AA subjects were assessed using the paired t test, taking $p < 0.05$ to indicate statistical significance. Genotype differences in indices of energy expenditure were adjusted for genotype differences in body composition using repeated measures analysis of variance.

Results

Adolescents with SS disease had similar socioeconomic scores, but were significantly shorter and lighter, with less LBM than their AA controls (Table 22). As expected SS subjects had a higher resting and flex HR (Table 22) and RMR than AA controls (Table 23). The standard deviation of differences between duplicate values of RMR was 0.5 MJ.d^{-1}.

Oxygen consumption values for treadmill exercises were obtained for 58/64 measurements. For the 11 SS/AA pairs with measurements for all 4 high activity levels, there was no difference in the fit of the regression of O_2 consumption on HR (median of mean sum of squares was 2.8 ml.min^{-1} for SS and 2.5 ml.min^{-1} for AA subjects; p=0.42 using Wilcoxson's test). In these 11 SS/AA pairs the mean (SD) slope of the regression line was 0.22 (0.13) in SS and 0.39 (0.10) in AA subjects (p<0.001 using paired t test).

As result of high energy expenditure above flex HR, TDEE was greater in AA controls (mean, SD: 13.8, 4.9 MJ.d^{-1}) than SS patients (10.5, 2.2 MJ.d^{-1}; p=0.034) (Table 24). The absolute energy spent in physical activity (TDEE minus RMR) and the percentage of time spent in high intensity activities (above 1.5 x Flex HR) were also greater in AA than SS subjects although the latter index just failed to reach statistical significance. The average physical activity level (PAL) (TDEE/RMR) in AA controls (mean, SD; 2.2, 0.8) was 45% greater than in SS patients (1.5, 0.3), the increase being highly significant (p=0.006) (Table 24).

Table 22

Comparison of Anthropometry, Haematology and Heart Rates of 16 SS Subjects with 16 AA Controls

	AA Mean	(SD)	SS Mean	(SD)	Significance p
Anthropometry					
Age (years)	18.3	(0.9)	18.0	(1.1)	0.007
Height (cm)	175.1	(4.8)	170.5	(4.0)	0.023
Weight (kg)	58.8	(4.3)	51.8	(4.5)	0.001
MAC (cm)	25.8	(1.1)	23.7	(2.0)	0.002
Triceps SF (mm)	5.5	(1.4)	6.2	(1.3)	0.21
Subscapular SF (mm)	7.9	(1.1)	6.9	(0.9)	0.028
BMI (kg/m^2)	19.2	(1.2)	18.0	(1.1)	0.014
LBM (kg)	56.7	(4.3)	49.1	(3.3)	0.001
Fat mass (kg)	2.1	(1.7)	2.7	(3.4)	0.038
SES	13.5	(4.2)	13.8	(3.2)	0.84
Haematology					
Haemoglobin (g/L)	142.0	(13.0)	87.0	(13.0)	<0.001
HbF (% Hb)[1]	0.2	(0.1-0.6)	1.9	(0.1-25.4)	0.15
Heart rates (beats/minute)					
Resting	52.4	(8.6)	64.2	(8.0)	0.001
Sitting	58.9	(11.9)	68.3	(9.5)	0.018
Standing	69.1	(13.4)	82.1	(13.6)	0.010
Flex	71.6	(10.8)	88.8	(10.9)	<0.001

MAC = mid-arm circumference;
SF= skinfold;
SES= Socioeconomic score (range: 0 -18);
BMI = Body mass index weight/height^2;
[1]median (range)

Table 23

Comparison of Energy Expenditure in 16 SS subjects with 16 AA Controls

Variable	AA controls (n=16) mean	(SD)	SS disease (n=16) mean	(SD)	Paired t test p
Sedentary					
Resting metabolic rate (RMR) (MJ.d^{-1})	6.3	(0.5)	7.0	(0.9)	0.018
Respiratory Quotient	0.81	(0.1)	0.89	(0.2)	0.17
Sitting MR (MJ.d^{-1})	6.7	(0.9)	7.3	(1.0)	0.049
Standing MR (MJ.d^{-1})	7.1	(0.9)	7.7	(1.1)	0.13
Sitting MR/RMR ratio	1.0	(0.1)	1.1	(0.1)	0.83
Standing MR/RMR ratio	1.1	(0.1)	1.1	(0.1)	0.62
[1]Activity (MJ.d^{-1})					
Level 1 (n=12)	17.0	(2.7)	16.4	(2.8)	0.69
Level 2 (n=16)	19.7	(4.9)	19.0	(3.6)	0.55
Level 3 (n=16)	32.1	(8.6)	26.6	(5.8)	0.04
Level 4 (n=15)	42.9	(11.9)	30.7	(5.8)	0.001
Level 1/RMR ratio	2.7	(0.4)	2.4	(0.5)	0.21
Level 2/RMR ratio	3.1	(0.8)	2.7	(0.5)	0.10
Level 3/RMR ratio	5.0	(1.4)	3.8	(0.8)	0.004
Level 4/RMR ratio	6.7	(2.0)	4.4	(0.8)	0.001

MR = Metabolic rate;

MJ.d^{-1}= Mega Joule per day;

[1]Activity levels 1-4 are walking at 1.5 km/hr with zero gradient, and at 1.5, 3 and 5 km/hr with a 10% gradient.

Table 24

Comparison of Free Living Energy Expenditure and Physical Activity Levels in 16 SS Subjects with 16 AA Controls

	AA Mean (SD)		SS Mean (SD)		Significance p
Time < Flex HR (%)	53.2	(17.5)	65.6	(20.1)	0.11
Time > 1.5x Flex HR[1](%)	3.1	(0-31)	0.1	(0-11.3)	0.06
Resting Metabolic Rate					
RMR (MJ.d^{-1})	6.3	(0.5)	7.0	(0.9)	0.018
Energy Expenditure (MJ.d^{-1})					
Low activity <Flex	3.6	(1.2)	4.8	(1.5)	0.033
High activity >Flex	10.2	(5.8)	5.7	(3.4)	0.022
Total daily (TDEE)	13.8	(4.9)	10.5	(2.2)	0.034
Physical Activity					
PAL (TDEE/RMR)	2.2	(0.8)	1.5	(0.3)	0.006
TEE - RMR (MJ.d^{-1})	7.5	(4.9)	3.5	(2.2)	0.01

MJ.d^{-1}= Mega Joule per day; PAL= Physical activity level;
[1] Median (range) given

Adjusting for genotype differences in weight reduced the genotype difference in TDEE from 3.3 MJ.d^{-1} (95% CI: 0.5 to 6.1) to 1.9 MJ.d^{-1} (95% CI: -2.2 to 6.1) (Table 25) but made little difference to the genotype difference in PAL, (from 0.7 MJ.d^{-1} [95% CI: 0.3 to 1.1] to 0.6 MJ.d^{-1} [95% CI: -0.06 to 1.2]) which then just failed to reach statistical significance. This method of adjusting for covariates tends to inflate standard errors making interpretation of significance levels difficult. However the correlation between genotype difference in weight and genotype difference in PAL was small (r=0.11, p=0.69). Adjusting for LBM also had a greater effect on the genotype difference in TDEE than on the genotype differences in PAL or energy expenditure of physical activity (TDEE - RMR) (Table 25).

In SS subjects RMR was not significantly correlated with percentage of time spent below flex HR (r=0.12, p=0.67) or percentage of TDEE spent in activities above flex HR (r= −0.16, p=0.54) and the corresponding figures for AA controls were similar (r=0.21, p=0.43 and r= −0.09, p=0.73). Between-subject variability in total energy expenditure and hence physical activity level was greater than within-subject variability seen on two different days and accounted for 78% of the total variance in AA controls and 87% in SS patients.

Net Mechanical Work Efficiency

The net mechanical work efficiency for walking at 5 km/hr on a 10% gradient was significantly greater in SS (median, [range]: 26%, [17 - 46]) than AA (19%, [12 - 41]) subjects (comparison by Mann-Whitney test: p=0.023). The corresponding figures for walking at 3 km/hr on a 10% gradient in SS (median, range: 18%, [13 - 40]) and AA (16%, [10 - 43]) adolescents did not differ significantly (p=0.23).

As expected, lighter SS subjects had a lower energy cost at any exercise (treadmill) activity than AA controls (Table 23). However when expressed relative to RMR the energy cost of higher intensity exercise at levels 3 and 4 was greater in AA than SS subjects.

Table 25							
Genotype Differences in Energy Expenditure after Adjusting for Genotype Differences in Body Composition							
	Mean genotype difference (95% Confidence Interval)						
	TEE (MJ.d^{-1})		RMR(MJ.d^{-1})		TEE/RMR		TEE-RMR(MJ.d^{-1})
Unadjusted	3.3	(0.5, 6.1)	-0.7 (-0.2, -1.2)		0.7 (0.3, 1.1)		3.9 (1.2, 6.7)
Adjusting for:							
Weight	1.9	(-2.2, 6.1)	-1.3 (-0.7, -1.9)		0.6 (-0.06, 1.2)		3.2 (-0.9, 7.2)
LBM	2.4	(-2.5, 7.3)	-1.4 (-0.7,-2.1)		0.7 (-0.03, 1.4)		3.8 (-0.9, 8.5)
Fat mass	3.7	(0.7, 6.8)	-0.7 (-0.13, 1.3)		0.8 (0.3, 1.2)		4.4 (1.5, 7.4)
LBM= Lean body mass; MJ.d^{-1}= Mega Joule per day							

Discussion

Minute by minute HR recording is a relatively simple and inexpensive method of measuring free living TDEE in small population groups (Kalkwarf *et al*, 1989). The technique compares favourably with TDEE estimated using activity diaries (Kalkwarf *et al*, 1989), whole body calorimetry (Spurr *et al*, 1988; Ceesay *et al*, 1989) and double labelled water (Schulz *et al*, 1989; Livingstone *et al* 1990) and is reasonably accurate provided that individual calibration curves are obtained for oxygen consumption against HR (Li *et al*, 1993). The relationship between energy expenditure and HR, although valid for high intensity exercise, may however not be accurate at light activity levels. Emotional rises in HR or venous pooling of blood can increase HR without a proportion change in energy expenditure and different curves have been fitted to energy expenditure/HR data in an attempt to improve overall accuracy (Schulz *et al*, 1989; Li *et al*, 1993). Another approach, as in the present study, is to use only the high activity part of the oxygen consumption/HR relationship to estimate energy expenditure above flex HR (Livingstone *et al*, 1990) and the mean oxygen consumption of resting activities to estimate energy expenditure for all time (including sleep) spent below flex HR. The accuracy of this method itself depends on the appropriateness of the flex HR value, although in the present study, visual inspection of VO_2/HR regression lines confirmed separation of high and low activities by a flex HR taken as the mean of HRs at resting activities and lowest exercise activity.

The use of the mean VO_2 of all resting (sedentary) activities to estimate all energy expenditure for HRs below flex (for both waking hours and sleep) is another potential source of error. This method is likely to slightly overestimate TDEE in both SS and AA subjects, but avoids errors in subjectively recording sleep times which vary from day to day and may occur randomly throughout the 24 hour period in Jamaica. Furthermore sedentary energy expenditure was similar to RMR in the present study and therefore substituting sedentary for sleep energy expenditure is likely to result in an approximate over-estimation of 5% in sleep

energy expenditure but a less than 2 % error in TDEE (Goldberg *et al*, 1988). Using HR to estimate TDEE also fails to include the thermogenic effect of food but, although there are no data for SS patients, this is likely to be a small component of TDEE in both AA and SS subjects (FAO/WHO/UNO 1985). Finally, calculation of energy expenditure from oxygen consumption using an assumed RQ, although another potential criticism, is unlikely to invalidate genotype comparison of TDEE as RQ is similar in patients with an SS or AA genotype.

Total daily energy expenditure comprises the energy requirements for basal metabolic rate, diet induced thermogenesis, energy cost of growth and energy expended in physical activity. Of all these components, the latter is most the variable and consequently may explain a large part of the inter-individual variation in energy requirements (Ravussin and Bogardus, 1989). In the present study, physical activity level was 30% lower in SS disease compared to AA controls who had PAL values in similar to those obtained from studies of healthy volunteers and consistent with moderate physical activity (Schulz *et al*, 1989). In contrast, the mean PAL index in SS adolescents was similar to light physical activity seen in sedentary subjects (FAO/WHO/UNO 1985). The doubling of energy spent above flex heart rate in AA compared to SS adolescents was consistent with a marked reduction in physical activity in SS disease and the low within- compared to between-subject variation in PAL suggested that this reduction was habitual and characteristic of patients. Adjusting for genotype differences in body size and composition appeared to reduce genotype differences in TDEE but had little effect on indices of physical activity.

The factors reducing physical activity in SS disease are unclear but as SS and AA adolescents were from the same cultural and socioeconomic backgrounds these variables are unlikely to account for these genotype differences. The presence of chronic disease and in particular anaemia could lead to behavioural changes and the avoidance of intense physical activity in some SS patients.

However, activities of daily living are unlikely to produce sustained periods of high intensity activity in either genotype and all SS patients in the present study were in the steady-state and entirely clinically well. Reducing physical activity is a compensatory mechanism in children with low energy intakes (Spurr and Reina, 1989; Torun 1990) and a similar adaptive response may occur in SS disease. Patients with SS disease could compensate for a high RMR by economising on physical activity, a mechanism observed in children with cystic fibrosis who also have a high RMR (Spicher *et al*, 1991). Furthermore, the energy cost of carrying out a specific task appears to be reduced in malnourished populations (Waterlow 1990) and the greater mechanical work efficiency and lower energy costs (expressed as a multiple of RMR) (FAO/WHO/UNU, 1985) in SS adolescents at high stress loads (5 km/hr) are consistent with a similar adaptation in SS disease. The mechanisms for increased mechanical work efficiency in undernourished populations are at present poorly understood but may be related to a behavioural adaptation similar to that in undernourished Gambian men (Minghelli *et al*, 1990).

Summary

The RMR of SS patients was greater than that of AA controls but TDEE, measured by the heart rate method was greater in AA controls (mean, SD: 13.8, 4.9 $MJ.d^{-1}$) than SS patients (10.5, 2.2 $MJ.d^{-1}$; p=0.034). The physical activity level (TDEE/RMR) was 45% greater in AA controls than SS patients and adjusting for genotype differences in body weight reduced the genotype difference in PAL from 0.70 (95% Confidence Intervals: 0.3 to 1.1) to 0.6 (95% CI: -0.06 to 1.2). Reducing physical activity is a compensatory mechanism in children with low energy intakes and a similar adaptive response may occur in SS disease.

IS THERE AN INFLAMMATORY RESPONSE IN STEADY-STATE SS DISEASE?

Reduced physical activity in adolescents is consistent with poor energy balance in SS disease although the mechanisms for a possible energy deficiency are unknown. Patients may fail to compensate for high resting expenditure by increasing oral intake and this relative anorexia is likely to deteriorate further during painful crises and infections. Mechanisms for inadequate energy intake in afebrile steady-state SS patients are unclear but one possibility is the anorexia produced by a diet deficient in specific nutrients relative to requirements (Kleiber 1945). Another possibility is the presence of subclinical inflammation leading to anorexia and this hypothesis was investigated in the present study.

The clinical course of sickle cell disease is punctuated by a variety of complications but even during the steady-state, chronic haemolysis continues and subclinical vaso-occlusion is presumed to occur. Other metabolic abnormalities reported in steady-state SS disease include an approximate doubling of protein turnover (Badaloo *et al*, 1989), an increase in urinary nitrogen loss (Odonkor *et al*, 1984), and a negative nitrogen balance on high nitrogen intakes (Odonkor *et al*, 1982b), observations suggesting inefficient nitrogen utilisation. Changes in other serum components include a significant depression of serum zinc (Prasad *et al*, 1976; Phebus *et al*, 1988), and elevation of copper (Peterson *et al*, 1975; Prasad *et al*, 1976) and of ferritin (Peterson *et al*, 1975), the latter occurring even in patients without histories of transfusion (Singhal - unpublished observations) and in patients with absent bone marrow iron stores (Peterson *et al*, 1975). These findings are all consistent with an acute-phase response and raise the possibility that inflammation occurs in SS disease even during the steady-state. This hypothesis was tested by measuring concentrations of the acute-phase reactants C-reactive protein (CRP), serum amyloid A (SAA), alpha-1-acid glycoprotein,

fibrinogen and plasma viscosity in patients with SS or SC disease while in the steady-state and in normal controls.

Subjects and Methods

A total of 258 cohort children participated in the study (143 with SS disease, 35 SC with disease and 80 AA controls) in five study groups. The first two groups were based on sera stored at $-70^{\circ}C$ for between 5-7 years at the time of assay, in whom review of the notes indicated the subjects to be clinically well. Group I included all available sera from SS subjects and AA controls when aged 8 years and group II when aged 10 years. Group III comprised SS patients and age/sex matched AA controls aged 15-17 years from whom sera were collected prospectively over a 6 month period on routine visits of clinically well subjects. Group IV comprised patients with SS disease attending the clinic during a 3 week period in whom the steady-state had been especially rigorously defined by excluding anyone with clinical problems in the preceding 2 weeks. Group V consisted of routine attendances during another 3 week period with similarly rigorous definition of steady-state but was expanded to include SS, SC, and AA genotypes.

CRP and SAA were measured in all groups but alpha-1-acid glycoprotein, fibrinogen concentration and plasma viscosity were also measured in group V. Sera samples were sent in dry ice to the laboratories of Dr. John Raynes (London School of tropical Medicine) where assays were conducted. CRP and SAA were assayed with a competitive inhibition, enzyme linked immunoabsorbent assay. In the absence of normal Jamaican data, the normal upper limit was assumed to be 10 mg/L for CRP (Gillespie *et al*, 1991) and arbitrarily taken as 5 mg/L for SAA. Alpha-1-acid glycoprotein was measured using immunoprecipitation with goat anti-human Alpha-1-acid glycoprotein and serum standards (Behring, Hounslow, Middlesex), and fibrinogen was assayed using immunoprecipitation with rabbit anti-human fibrinogen (Dako, High Wycombe, Bucks, code no. A080) according

to Dako application notes, both procedures being performed on a Cobas Fara analyser. Plasma viscosity was determined using a Coulter Harkness viscometer (Coulter Electronics Ltd, Luton, UK).

CRP and SAA concentrations were markedly skewed and there was no satisfactory transformation. Results were therefore presented as the median and range of the distribution and the nonparametric Mann-Whitney test used to assess the significance of the difference between genotypes in groups I, II and V. In Group III the genotype difference was tested by the Wilcoxon matched-pair test.

Results

The distributions of CRP and SAA concentrations in all study groups are summarised in Table 26 and the distribution of the other acute-phase reactants measured in group V in Table 27. Compared to AA controls, concentrations in SS disease were higher for CRP in groups II, III and V, and for SAA in group III. The relationship between serum concentrations of CRP and SAA in individual patients and controls from group V are presented in Figure 9.

In all groups combined, CRP concentrations exceeded 10 mg/L in 43/143 (30%) of SS subjects compared to 9/80 (11%) of AA controls, and SAA levels exceeded 5 mg/L in 45/143 (31%) SS subjects and 5/80 (6%) of controls. Both CRP and SAA were elevated in 27/143 (19%) of all subjects with SS disease, in 6/35 (17%) of subjects with SC disease and in 1/80 (1%) of AA controls. In group V where four indices of the acute-phase response were measured, an attempt was made to determine whether the same individuals manifested differences in all indices. Among the SS subjects, 14/52 (27%) showed increased levels of both CRP and SAA (group A) whereas 24/52 (46%) showed both levels within the normal range (group B). The distribution of fibrinogen levels in group A (median, range; 4.0, 3.1–5.7 g/L) did not differ from those in group B (3.8, 2.7–5.8 g/L) (p=0.31), whereas plasma viscosity was significantly higher in group A (median, range; 1.81, 1.58–1.93 mPa.s) compared to group B (1.67, 1.56–1.88 mPa.s)

(p=0.023). There is therefore evidence to suggest that patients with high CRP and SAA concentrations tend to have a higher plasma viscosity than patients with low CRP and SAA. The correlations between different acute-phase reactants and plasma viscosity are shown in Table 28.

Table 26

C- reactive Protein and Serum Amyloid A in Steady-state Sickle Cell Disease

Study group	Genotype	n	CRP Median (range) (mg/L)	p	SAA Median (range) (mg/L)	p
I	AA	16	4.1 (1-54)	0.073	0.6 (0.5-110)	0.48
	SS	16	1.0 (1-160)		1.0 (0.5-80)	
II	AA	30	1.0 (1-14)	0.041	0.8 (0.5-5.9)	0.087
	SS	34	2.0 (1-160)		1.5 (0.5-13.5)	
III	AA	19	1.0 (1-3.9)	0.0096	0.6 (0.5-4.5)	0.017
	SS	19	2.8 (1-42)		1.1 (0.5-40.0)	
IV	SS	22	2.2 (1-64)	-	3.8 (0.5-50.0)	-
V	AA	15	4.0 (1-30)	0.025*	3.0 (0.2-23.0)	0.50*
	SS	52	9.0 (1-100)	0.16⁺	3.5 (0.5-65.0)	0.46⁺
	SC	35	8.0 (2-120)		4.0(1.0-31.0)	

Comparison of AA and SC with SS determined by Mann-Whitney test for Groups I,II and V and Wilcoxon's matched pairs for Group III.
*AA vs SS; ⁺ SC vs SS
SAA= Serum Amyloid A
CRP= C-reactive protein

Table 27

Acute-phase Reactants Measured only in Study Group V

| Genotype	Alpha-1-acid glycoprotein (mg/L) Median (range)	p	Fibrinogen (g/L) Median (range)	p	Plasma viscosity (mPa.s) Median (range)	p
AA (n =15)	620 (350-1140)	0.68*	3.95 (2.8-5.1)	0.88*	1.66 (1.52-1.79)	0.088*
SS (n = 52)	670 (400-1340)	0.62$^+$	3.90 (2.1-11.8)	0.02$^+$	1.69 (1.52-2.05)	0.058$^+$
SC (n = 35)	640 (380-1000)		3.60 (2.5-5.0)		1.66 (1.51-1.90)	

* AA vs SS; $^+$SC vs SS

Table 28

Spearman Correlation Matrix for Acute-phase Reactants in AA, SC, and SS Subjects

	CRP	SAA	AGP	Fibrinogen	Plasma viscosity
SAA	0.54* 0.41* 0.45**				
Alpha-1-acid glycoprotein (AGP)	0.46 0.32 0.46**	-0.01 0.35* 0.05			
Fibrinogen	0.30 0.27 0.33*	-0.38 0.15 -0.05	0.50 0.53** 0.40**		
Plasma viscosity	0.47 0.29 0.32*	0.22 0.25 0.27	0.80** 0.38* 0.37**	0.61* 0.49** 0.51***	
Neutrophils	-0.23 0.07 0.02	-0.22 0.32 0.37**	0.17 0.24 0.02	0.27 0.19 -0.09	0.37 0.54** 0.28*

Top line is for AA (n=15), middle line for SC (n=35), and bottom for SS (n=52) subjects
*p < 0.05, **p < 0.01, ***p < 0.001;   AGP= alpha-1-acid glycoprotein

Figure 7: **Relation Between C-reactive Protein (CRP) and Serum Amyloid A**

(SAA) in SS and SC disease and in AA controls
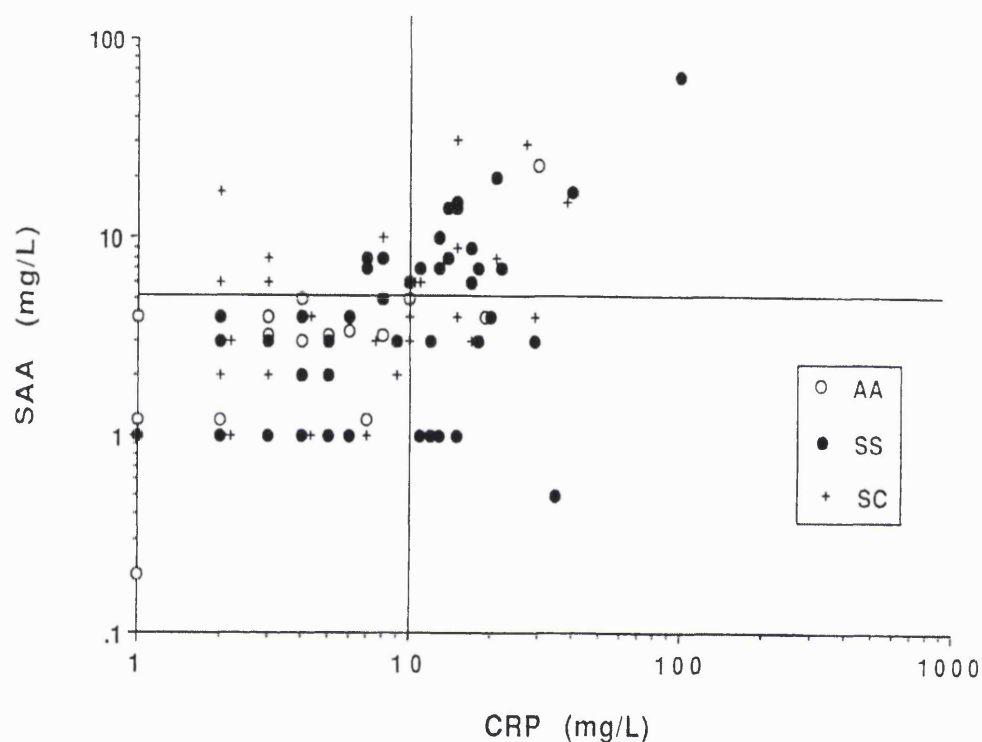
To investigate the role of haemolysis in these abnormalities levels of acute-phase proteins and plasma viscosity were correlated with indices of haemolysis in SS subjects in Group V. This analysis showed no significant correlations between any acute-phase index and total haemoglobin, fetal haemoglobin, absolute reticulocyte count, bilirubin level, or platelet count. The proportional reticulocyte count was correlated with SAA only (r=0.28, p=0.047).

Discussion

There have been few published studies of acute-phase reactants in steady-state SS disease. Becton *et al* (1989) reported that the mean levels of the acute-phase

reactants CRP, C3 complement, and alpha-1-antitrypsin were within the normal range in children on routine clinic visits but became elevated during the painful crisis or bacterial infection. Akinola *et al* (1992) found normal mean steady-state levels of CRP, alpha-1-acid glycoprotein, and fibrinogen, but serial CRP levels within individuals showed considerable variation with occasional elevated values. The present study has shown that CRP and SAA levels are more commonly elevated in SS and SC disease than in AA controls. The difference was not consistent in all groups, high CRP concentrations occurring in some AA controls in Group I, illustrating the difficulties in retrospective determination of an asymptomatic state. However even the rigorously and prospectively defined SS subjects with no known pathology in the preceding 2 weeks had elevated concentrations of either CRP or SAA in 50% of Group IV and 54% of Group V.

Since an elevated CRP is considered evidence of active tissue damage (Pepys 1981), these findings support the concept of an intermittent inflammatory process in SS disease even during asymptomatic periods. Significant correlations between CRP levels and other acute-phase reactants are consistent with this hypothesis. Red cell deformability also varies in serial observations in steady state individuals (Akinola *et al*, 1992; Lucas *et al*, 1985), some measurements showing a loss of deformability similar to that observed in the painful crisis. This observation is also consistent with an acute-phase response resulting from sub-clinical micro-infarction.

Summary

The concentrations of the acute-phase reactants, C-reactive protein and serum amyloid A protein were increased above 10 mg/L and 5 mg/L respectively in 19% SS, 17% SC, and in 1% of AA subjects. It is postulated that subclinical vaso-occlusion may generate a covert inflammatory response which may contribute to the growth and metabolic abnormalities in SS disease.

CHAPTER 4: CONCLUSION AND FUTURE STUDIES

The Pattern of Growth

Retarded growth is common in children with SS disease. A progressive deficit in height and weight appears to emerge at around 6 months of age and skeletal maturation is retarded from as early as age 5 years (Stevens *et al*, 1986). Weight appears to be impaired more than height (Luban *et al*, 1982; Platt *et al*, 1984) and boys may be more severely affected than girls (McCormack *et al*, 1976; Phebus *et al*, 1984). Patients may also have differences in body shape, thinner skinfolds, reduced mid arm circumference (McCormack *et al*, 1976; Stevens *et al*, 1983) and delayed adolescent growth.

The factors affecting growth are unclear but several studies suggest increased metabolic demand in SS disease (Badaloo *et al*, 1989, Odonkor *et al*, 1982a). Resting metabolic rate may be increased by 20% and protein turnover is almost doubled (Badaloo *et al*, 1989). Nitrogen balance may be difficult to maintain at high nitrogen intakes and the metabolism of urea is altered, some changes being similar to normal patients whose protein intake is insufficient to meet demand (Jackson *et al*, 1988). The mechanisms for these changes are not known but may be secondary to a high rate of erythropoiesis in SS disease.

Determinants of RMR

Erythropoiesis

Reduced red cell survival and subsequent increase in erythropoietic activity may increase RMR in SS disease. The fall in RMR after splenectomy for hypersplenism in SS children (Badaloo *et al*, 1996) and after suppressing erythropoiesis by blood transfusions in patients with beta thalassaemia (Vaisman *et al*, 1995) suggests that erythropoiesis has a significant energy cost. However, RMR in the present study did not correlate with serum TfR levels suggesting that increased erythropoietic

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mass was not a determinant of the increase in RMR in SS disease possibly because a higher turnover of haematopoetic cell lines other than erythrocytes contributed to the increase in basal metabolism. Confounding factors such as the energy cost of a high cardiac output could have also obscured any relationship between RMR and erythropoiesis although RMR did not correlate with Hb concentration or heart rate, both determinants of cardiac output. The role of erythropoiesis in increasing RMR therefore remains uncertain but could be resolved in future studies by RMR measurements in SS patients during episodes of altered haematopoiesis (eg. post blood transfusion or in an aplastic crisis) or in patients with SC disease who have a moderate increase in erythropoietic activity that overlaps with both SS and AA genotypes.

Body Composition

Genotype differences in body composition could explain the increase in RMR in SS disease. The RMR of any individual depends on the mass of metabolically active tissue and is usually expressed relative to LBM. However, LBM consists of tissues with different rates of metabolic activity, visceral tissues having higher metabolic rates than muscle. The relative mass of visceral and muscle tissue changes with physical maturation and the fall in RMR relative to body weight with increasing age is secondary to slower growth rates of visceral organs with high metabolic rates (brain, liver, heart and kidney) relative to those with lower metabolic rates (muscle and fat). These changes in body composition may be particularly important in studies of SS adolescents who have slower physical maturation than AA controls and higher RMR in SS adolescents may therefore reflect their greater visceral mass relative to muscle mass. Although SS and AA adolescents were all post-pubertal, adjusting for the higher non-muscle (visceral) to muscle mass ratio of SS patients reduced their increase in RMR from 22% to 8% which then became statistically non-significant. Differences in body composition partly explained the increase in RMR in SS disease but studies with larger sample sizes would be required to detect any increase in RMR independent of genotype

differences in body composition. However, RMR measurements in patients at birth (before the increase in red cell turnover) and again at age 6 months (before genotype changes in body composition) could also help determine the relative contributions of body composition and red cell turnover to the increase in RMR of SS patients.

Protein Turnover

Two basic biochemical processes, protein synthesis and ion pumping are estimated to account for 2/3 or more of basal metabolism (Grande 1980) with the contribution of different organs to RMR depending on their relative activity of these processes. The contribution of bone marrow activity to RMR is unknown but an increase in haemoglobin synthesis from 6.25 g/day in normal adults to 40 g/day in SS disease may increase protein turnover and hence RMR. Protein turnover is approximately doubled in SS disease, accounting for up to 37% of the RMR of SS patients (Badaloo *et al*, 1989), compared to only 15-20% in healthy AA adults (Waterlow 1990). Furthermore the 30% fall in protein turnover (2.3 $g.kg.^{-1}d^{-1}$) (Badaloo *et al*, 1996), 15% fall in RMR (34 $KJ.kg^{-1}.d^{-1}$), 60% fall in reticulocyte counts, and doubling of Hb concentration after splenectomy for hypersplenism is consistent with increased erythropoiesis contributing to higher RMR and protein turnover in SS disease. However, improvements in Hb concentration or reticulocyte count did not correlate significantly with changes in protein turnover or RMR in this study (Badaloo *et al*, 1996) suggesting that the effect of erythropoiesis on metabolism in SS disease requires further investigation. Further, assuming an energy cost for protein turnover of 9 $KJ.kg^{-1}.d^{-1}$ (Badaloo *et al*, 1989), the fall in protein turnover accounted for only 62% of the fall in RMR post-splenectomy (Badaloo *et al*, 1996), consistent with more than one mechanism contributing to the elevation of RMR in SS disease.

The doubling of protein turnover in SS disease is estimated to be greater than the theoretical increase resulting from the higher turnover of haemoglobin alone and may be secondary to an increase in turnover of cellular components of

erythropoietic tissue other than haemoglobin or to genotype differences in body composition. A greater proportion of visceral mass to muscle mass explains the higher protein turnover per kg of LBM of malnourished compared to well-nourished adults (Soares *et al*, 1991) and a similar mechanism could contribute to higher protein turnover per kg body weight of SS patients who have a lower body mass index than their AA controls.

Increased protein turnover in SS disease may also reflect inefficient nitrogen utilisation and high rates of urea production and negative nitrogen balance on high nitrogen intakes (Odonkor *et al*, 1982b) in SS patients are consistent with this hypothesis. Correction of this inefficiency could reduce both protein turnover and RMR and the fall in protein turnover after splenectomy for hypersplenism (Badaloo *et al*, 1996) could occur either directly by decreasing red cell turnover or by improvements in metabolic efficiency. The proportion of protein turnover attributable to bone marrow activity in SS disease is uncertain but likely to be high and could be investigated in the future by determining red cell and protein turnover in models in which the bone marrow activity deviates markedly from normal steady-state levels such as in the aplastic crisis. N^{15} labelled glycine studies measuring protein turnover specifically in the bone marrow compartment (London *et al*, 1949) may also help to indicate the extent of the increase in protein turnover directly attributable to an increase in effective erythropoiesis.

Is there a Nutrient Deficiency in SS disease ?

The pattern of growth in children with SS disease (low weight and height, reduced skinfold thicknesses, and a delay in skeletal maturation and pubertal development) is consistent with chronic childhood malnutrition (Eveleth 1985; Reed *et al*, 1987). Birth weight is similar in SS and AA babies but growth deficits in SS children emerge at 6 months of age (Stevens *et al*, 1986) coinciding with the fall in HbF, increase in red cell turnover and therefore a possible increase in nutritional requirements. The growth response to nutritional supplementation is consistent

with a marginal nutritional state (Heyman *et al*, 1985) and suggests that dietary intake in SS patients may be inadequate to meet their higher metabolic demands Which nutrients may be deficient in SS children is not known but the concept of relative malnutrition in SS disease is supported by other metabolic abnormalities reminiscent of normal subjects on low protein diets such as abnormal urea kinetics (Jackson *et al*, 1988) and increased excretion of orotic acid (a measure of arginine deficiency, Enwonwu *et al*, 1990). Poor growth, reduced skinfold thickness and fat deposits suggest a marginal energy balance in SS children and, although the mechanism of this deficiency is unclear, both low energy intake and high energy expenditure are likely to contribute.

There is a dearth of formal dietary information in SS disease. Published data suggest that energy intake in SS patients is similar to that of AA controls (Phebus *et al*, 1988, Tangney *et al*, 1989). However, these studies can be criticised for failing to match patients and controls for socioeconomic status and using methods to estimate habitual food intake that have systematic errors of the same order of magnitude as likely genotype differences in nutrient intake. Furthermore all studies of dietary intake in SS disease have studied only steady-state SS patients, thereby omitting the anorexic effects of recurrent painful crises and febrile illness. The more accurate 3 or 5 day weighed intake method could detect any large genotype differences in dietary intake but may fail to detect a small dietary deficiency which could have a cumulative effect on nutritional status. Poor compliance and technical difficulties made a 3 day weighed dietary intake impractical in Jamaica, but future studies using double labelled water to determine metabolisable energy intake combined with RMR and physical activity measurement to estimate energy expenditure could assess energy balance in SS disease.

It is not known whether a suboptimal energy intake in SS disease is related to an unavailability of calories or inadequate voluntary energy intake. The failure of oral supplementation and effect of nasogastric nutritional supplementation in

improving growth of SS boys (Heyman *et al*, 1985) suggests that the latter mechanism predominates. However, the greater effect of socioeconomic status on the growth of SS compared to normal AA subjects (Knight, personal communication) suggests a contribution from the availability of calories. Failure to increase energy intake to match demand raises the possibility of appetite suppression in SS disease and a low grade chronic inflammatory process could be the underlying mechanism for this relative anorexia. Metabolic changes and anorexia are presumed to result in part from chronic inflammation in Crohn's disease (Kelts *et al*, 1979), cystic fibrosis (Kraemer *et al*, 1978), and *Trichuris* dysentery (Cooper *et al*, 1990) all of which manifest anthropometric abnormalities and growth retardation similar to those in SS patients. Investigation of the role of the inflammatory response and of the cytokine mediators of inflammation in energy balance (Grimble 1990) could therefore contribute to the understanding of suboptimal nutrition and growth failure in SS disease.

A deficiency of nutrients other than energy could also occur in SS patients. The metabolic demands for erythropoiesis may require an unusual balance of amino acids and particularly glycine which is required for synthesis of the haem ring. Although glycine can be readily synthesised as a non-essential amino acid, high glycine requirements in SS disease may exceed dietary intake and the capacity of synthesis, leading to a relative glycine deficiency. Increased urinary excretion of 5-oxoproline (an index of glycine deficiency, Jackson *et al*, 1987) is consistent with a marginal balance of glycine in SS disease and as glycine requirements are also high during periods of rapid growth (Persaud *et al*, 1987), competing demands of erythropoiesis and growth for glycine could contribute to growth failure in SS children. It is of interest therefore that the acceleration in growth following splenectomy for hypersplenism affects height and not weight, consistent with a deficiency of a specific nutrient such as glycine rather than a general improvement in nutritional state. Measuring urinary 5-oxoproline (an index of glycine

deficiency) in hypersplenic SS patients before and after correction of hypersplenism by splenectomy may therefore help to clarify the role of glycine deficiency.

The possibility of a specific nutrient deficiency generates other hypotheses for the mechanisms of growth and metabolic abnormalities in SS disease. A specific nutrient deficiency could be growth limiting in children, causing all other components in excess of requirements to be used inefficiently and requiring energy for their metabolism and safe excretion. This mechanism could contribute to the increase in RMR in SS disease and also suppress appetite (Kleiber 1945), leading to an energy deficiency. An unusual balance of amino acid requirements, particularly of glycine, could be further exacerbated in SS disease by the requirements for synthesis of acute-phase proteins (Grimble 1990), making the growth of SS children particularly sensitive to the quality of protein intake.

The Consequences of Undernutrition

Growth

Faced with higher metabolic demands, children with SS disease may economise on energy expenditure by reducing growth. Although energy requirements for growth are small relative to total energy consumption (15-30% at birth, falling to only 5% at one year of age (FAO/WHO/UNU, 1985), growth is an early casualty of calorie economy. A chronic energy deficiency may force a resetting of priorities and SS children may adapt to adverse environmental circumstances by prolonging the growth period, similar to adaptive mechanisms seen in chronically malnourished children. The observation that final height is maintained suggests a relatively mild growth retarding insult or that the delay in epiphyseal fusion is a consequence of SS disease of unknown mechanism.

Competition for essential calories and nutrients from erythropoiesis and growth plates could impair growth in SS children, an hypothesis currently supported by several observations. High levels of fetal haemoglobin which reduce the haemolytic and therefore the erythropoietic rate, are associated with more

normal growth and stimulating HbF production with hydroxyurea is associated with increased weight gain in SS adults (Charache *et al*, 1992). Further, excessive haemolysis, consequent on hypersplenism, reduces growth and correction of hypersplenism by splenectomy is followed by a growth spurt and a fall in RMR (34 $KJ.kg^{-1}.d^{-1}$), (Badaloo *et al*, 1996) that represents an energy saving in excess of that required for the normal rate of growth of a one year old child (22 $KJ.kg^{-1}.d^{-1}$, for deposition of 1 $g.kg^{-1}.d^{-1}$ balanced new tissue; Spady *et al*, 1976). However, the growth of a heterogeneous population such as children with SS disease is likely to be affected by many complex environmental factors other than erythropoiesis and it was not surprising therefore that the present study did not find an association between growth and a single variable such as serum TfR concentration. Future studies would need to measure confounding variables such as dietary intake, disease severity and socioeconomic status to get a clearer picture of the determinants of growth failure.

It is of interest that the protective effect of high HbF against poor growth was confined to boys. Observations that the beneficial effect of HbF on many complications of SS disease are confined to males have also been previously reported and raises the possibility that a low HbF level further increases erythropoietic and metabolic activity which may already be greater in SS boys than girls. This hypothesis is supported by the greater resting metabolic rate, irreversibly sickled cell counts (a determinant of haemolysis) (Mason *et al*, 1982), and serum TfR concentrations and poorer growth (Phebus *et al*, 1984) of boys than girls with SS disease. The effects of growth retardation are also likely to be more severe in SS adolescent boys who are at particular risk of psychological problems (Hurtig and Park, 1989), depression (Hilton *et al*, in press) and of osteoporosis in adult life (Finkelstein *et al*, 1992).

Physical Activity

Faced with higher RMR, SS patients have the options of increasing calorie intake or reducing energy expenditure for physical activity, a mechanism observed in

malnourished children and children with cystic fibrosis (Spicher *et al*, 1991). Few data are available on the physical activity of SS patients, but the present study suggests either the availability of calories or a poor appetite prevents an energy intake sufficient to maintain physical activity. In the short term, reducing physical activity may maintain energy balance but a persistent energy deficiency may be detrimental to optimal health (Torun 1990) by restricting activities such as competitive sport and manual work, and by retarding growth.

Psychosocial

A marginal energy balance may also effect cognitive development. A lowered intelligence quotient has been repeatedly observed in SS disease and attributed to a loss of schooling or other effects of a chronic disease process. A recent Jamaican study also found lower intelligence quotient in SS compared to AA adolescents which could not be accounted for by genotype differences in school attendance, disease severity, or a variety of social factors (Knight *et al*, 1995). The only independent factor significantly associated with intelligence quotient (measured at 15-18 years) was poor linear growth at all ages but discernible as early as one year of age.

Physical and mental development in otherwise normal children is impaired by suboptimal nutrition in the first year of life (Lucas *et al*, 1989) and has been shown to improve with nutritional supplementation in normal children (Waber *et al*, 1981, Walker *et al*, 1992), in stunted Jamaican children (Grantham-McGregor *et al*, 1991), and in children with thalassaemia major (Fuchs *et al*, 1996). The possibility of a similar effect of nutritional supplementation on both physical and mental development of children with SS disease has not been investigated. If a beneficial effect can be demonstrated, such supplementation would be an economically feasible intervention reducing the potential morbidity of this disease worldwide. Furthermore, this may be achieved in countries with limited financial resources where so much of the disease occurs.

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