LINEAR GROWTH RETARDATION AND STRESS LEVEL IN SCHOOL-AGED URBAN JAMAICAN AND NEPALI CHILDREN

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Abstract

Growth retardation (stunting) is a major public health problem in developing countries, where 38% of children under 5 years old are short for their age, and many have poor levels of mental development along with behavioural abnormalities. Animal research suggests that an altered stress response may contribute to the negative outcomes associated with undernutrition. This dissertation research investigated the associations of stunting, stress physiology (hypothalamic-adrenal and autonomic nervous system activity) and behaviour. Study 1 compared 30 stunted children with 24 non-stunted children, all of whom were participants in a prospective, longitudinal case-control study of children who were stunted in early childhood. Study 2 compared 31 stunted children with 31 non-stunted children, all of whom were newly recruited for the study. All children in both studies were 8-10 years old and lived in the same poor areas of Kingston, Jamaica. A test session of physiological and physical stressors was administered, and baseline and response levels of salivary cortisol, heart rate, and urinary catecholamines (epinephrine, norepinephrine, dopamine) were measured. Behaviours were observed during an interview and frustrating task. As compared with the non-stunted children, stunted children had significantly higher salivary cortisol levels, heart rates, and urinary epinephrine levels. Stunted children also vocalized less, were more inhibited, less attentive, and more frustrated than non-stunted children. After controlling for birthweight or social background, maternal and child IQ, the differences in cortisol level, heart rate, epinephrine, and inhibition remained significant. Study 3 compared 64 stunted with 64 non-stunted school-children from the same poor areas of Kathmandu, Nepal. A modified version of the Jamaican test session was administered, and levels of cortisol and heart rate were measured during testing and also during a baseline. Stunted Nepali children showed a blunted physiological response to psychological stressors, but were not different from the non-stunted children in baseline measures. These findings suggest that childhood growth retardation is associated with changes in physiological arousal, and that the relationship may be mediated by several socio-cultural, environmental, and physical variables.

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Dedication

In memory of my grandmother Catherine Graf Fernald (1914-1996)

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List of Abbreviations

ANCOVA: analysis of covariance

ANOVA: analysis of variance

ATP: adenosine triphosphate

BMI: body mass index

CI: confidence interval

CV: coefficient of variance

DQ: developmental quotient

ECG: echo-cardiogram

HPLC: high performance liquid chromotography

IQ: intelligence quotient

IUGR: intra-uterine growth retardation

LBW: low birthweight

MFFT: Matching Familiar Figures test

MHPG: methoxyhydroxyphenylglycol (3-methyl-4-hydroxyphenylglycol)

NBW: normal birthweight

NS: not significant

PEM: protein energy malnutrition

PPVT: Pearson Picture Vocabulary Test

RIA: radio-immuno assay

SGA: small for gestational age

VMA: vanillylmandelic acid

Chapter 1: Introduction

Childhood malnutrition is a public health problem of global proportions, particularly in developing countries. Stunted children have numerous developmental difficulties, including deficits in cognitive functioning and poor school performance. Research with animals has demonstrated a link between early malnutrition and heightened emotional reactivity, leading to the hypothesis that stress could interfere with cognitive and behavioural functioning. However, there has been almost no research with humans exploring this possibility.

In this doctoral research, I investigated the role of impaired stress response as a potential mediating variable contributing to the negative developmental outcomes in malnourished children. The present work evaluates the hypothesis that children who have experienced malnutrition (early childhood and concurrent) are less able to adapt to novel environments, and become frustrated and anxious much more easily than adequately nourished children. To address this question, the stress response of undernourished and adequately nourished children was compared using behavioural measures in combination with physiological measures of the cardiovascular, adreno-cortical and autonomic nervous systems.

Chapters 2-4 provide the theoretical context in which to set the thesis. Specifically, Chapter 2 reviews the effects of poor nutrition on development, Chapter 3 addresses linear growth retardation and Chapter 4 covers stress physiology. Following the introduction, the thesis primarily concerns two studies completed in Jamaica, and the conclusions and interpretation of those studies.

Chapter 5 outlines the aims and hypotheses for the studies, Chapter 6 outlines the methodology used, and Chapter 7 discusses the results from the physiological and behavioural data. Chapter 8 reviews results from an interview addressing problem situations and punishment in the life of Jamaican school children. Chapter 9 is the discussion and conclusions from the Jamaican studies.

All parts (aims, methods, results, discussion) of a much smaller, replication study undertaken in Nepal are discussed in Chapter 10. Although the Nepali study was designed to replicate the Jamaican work, it soon became clear that the studies would have to be treated differently, due to substantial cultural differences. Nonetheless, Chapter 11 is devoted to general conclusions that can be drawn by looking at the Jamaican and Nepali studies all together.

Parts of this dissertation have been published elsewhere:

Fernald, L.C. and Grantham-McGregor, S.M. Effects of early childhood undernutrition on stress response. Transactions of the Royal Society of Tropical Medicine and Hygiene 91: 500 (1997)

Fernald, L.C. and Grantham-McGregor, S.M. Stress response in children who have been growth retarded since early childhood. American Journal of Clinical Nutrition Sept. 68(3): 691-8 (1998).

Chapter 2: Effects of poor nutrition on development: an overview of the major issues in developing countries

Undernutrition: an overview

Introduction

Undernutrition is a critical problem for children living in developing countries, and can result in poor developmental outcomes, all of which are not yet fully understood. One clear and recurring finding is that poor nutritional status, in the form of energy, fat or micro-nutrient deficits, can contribute to reduced cognitive function and altered behaviour. This chapter addresses the major nutritional deficiencies (severe protein-energy malnutrition, low birth weight, fatty acids/ breastfeeding, iron and iodine deficiency) and their impact on poor development. These factors are reviewed because they may impact linear growth retardation (stunting) directly, and may therefore have mechanistic implications. Chapter 3 will directly and thoroughly addresses the problems of stunting because that is the focus of the thesis.

The impact of any particular nutritional deficiency depends on the stage of a child's development, as well as the severity and duration of the insult. In any case, the problems resulting from one nutritional deficit may be exacerbated by the presence of another, and the effects can be cumulative because deficiencies frequently occur together. In addition, nutritional insults often coincide with infections, such as gastro-enteritis, respiratory and geohelminths infections, which may add to the negative impacts of poor nutrition. The poor socio-cultural environments and many deprivations and disadvantages that occur in the homes of

undernourished children could themselves be detrimental to intellectual development (Richardson, 1974).

Since nutritional deficiencies nearly always occur in the presence of psycho-social and economic deprivations that could independently limit a child's development, demonstrating a direct causal link between poor nutrition and poor development is difficult. In order to make a claim of causality, a randomized controlled treatment trial is required in which a particular nutrient was shown to benefit a child's development.

Though it is not necessarily theoretically desirable to establish independent causality due to the increasing evidence that environmental and biological conditions are related in complex ways, many studies have isolated particular nutritional insults in order to evaluate their effects on mental and behavioural development.

Environmental correlates of poor nutrition

Many studies (Grantham-McGregor, 1984) have shown that malnourished children usually come from poor socio-cultural environments and suffer from a variety of factors. These include poor physical resources, such as over-crowded homes with poor sanitation and water supply, few household possessions and low income. Malnourished children usually come from unstable families, with large numbers of closely spaced children. Parents of malnourished children often have limited education and intelligence, and low skilled occupations. Stimulation in the home is generally poor, with few toys or books, and infrequent participation by the parents in play activities.

Effects of nutrition on mental and behavioural development

Severe protein-energy malnutrition

According to the 1999 State of the World's Children, 19% of children under

five in South Asia and 10% of children in Sub-Saharan Africa were severely

underweight from 1990-97, according to weight for age criteria. In Jamaica, 1% of

the under-fives is severely underweight, compared with 16% in Nepal. Although the

prevalence of severely underweight children (weights below -2SD of the reference)

is estimated to have declined globally from 38% in 1980 to 34% in 1990, the

absolute number of children has actually increased over the same period from 164 to

184 million (United Nations Subcommittee on Nutrition 1992). The most recent

estimates claim that 190 million children under five are chronically malnourished

(UNICEF, 1998). The prevalence of malnutrition is still very high in sub-Saharan

Africa, where drought and armed conflict has led to severe famine, with children in

refugee camps and displaced populations at increased risk.

Historical context

The earliest documentation of kwashiorkor has been traced back to a

Mexican publication from 1865. However, the syndrome was not formally named

and characterized until 1935. By the 1950's, protein deficiency was identified as the

primary cause of kwashiorkor. Yet, as research continued, it soon became evident

that children who developed kwashiorkor had insufficient intakes not just of protein,

but also of energy. In 1959, Jelliffe created the term protein-calorie malnutrition

(PCM), or protein-energy malnutrition (PEM) to classify kwashiorkor, along with

marasmus, and milder forms of malnutrition. Although there have been propositions

to replace the term protein-energy malnutrition with "energy-nutrient malnutrition,"

PEM is still the standard term in use.

In nearly all studies of severe malnutrition, the Gomez or Wellcome classifications of malnutrition have been used. These do not define specific diseases, but describe a mixture of clinical signs with differing aetiologies. Along with protein and energy deficiencies, a variable amount of infection, as well as many other types of deficiencies such as zinc, manganese, copper and iron may also be present. These may affect growth and behaviour differentially (Grantham McGregor et al. 1987). Thus, any interpretation of the literature is constrained by combining a heterogeneous group of conditions into inappropriate categories. The predominance of certain signs of malnutrition, or nutrient deficiencies may vary according to geographical area.

There has been much discussion as to whether children who suffered from different types of protein energy malnutrition (PEM) in early childhood subsequently show differing levels of mental development. There is some suggestion that children who suffer from kwashiorkor are less likely to have a deficit than those who suffer from marasmus. While the diagnosis of all forms of PEM depends on the presence of some degree of weight deficit, the diagnosis of kwashiorkor and marasmic kwashiorkor hinges on the presence of oedema (Lancet, editorial 1970). Waterlow and Rutishauser (1972) offered an alternative way of clasifying PEM by the degree of wasting or stunting. Wasting (weight expressed as percentage of expected weight for their height) indicates current nutritional status, and stunting (height expressed as percentage of expected height for age) indicates nutritional status over a longer period. They also observed that, in general, children with kwashiorkor are less stunted than those with marasmus. It is possible that the duration of malnutrition and hence the degree of stunting is more highly related to children's mental development than to short-term severity.

<u>Case control studies</u>

Most studies of severe malnutrition and mental development have concerned children who were hospitalized with marasmus, kwashiorkor, or marasmic kwashiorkor. On recovery from the acute stage and immediately after, children show poor developmental levels compared with matched controls or siblings (Pollitt and Granoff, 1967; Brockman and Ricciuti, 1971; McLaren et al. 1973; Grantham McGregor et al. 1987).

Cognitive deficits and motor skills

Many studies have been conducted to establish whether these negative effects remain at school age. In an attempt to control for malnourished children's poor socio-economic circumstances, they have been compared with carefully matched controls (Champakam et al. 1968; Hertzig et al. 1972; Hoorweg, 1976; Nwuga, 1977; Galler et al. 1984; Galler et al. 1984; Galler et al. 1983; Galler et al. 1983; Grantham McGregor et al. 1994) and/or siblings (Evans et al. 1971; Birch et al. 1971; Hertzig et al. 1972; Nwuga, 1977; Bartel et al. 1978; Pereira et al. 1979; Graham and Adrianzen, 1979; Moodie et al. 1980).

In most studies which used matched controls, differences favouring the controls have been found in tests of IQ and cognitive function. Studies involving siblings have had somewhat less consistent findings. Although differences have been found in some sibling comparisons (Birch et al. 1971; Hertzig et al. 1972; Nwuga, 1977), none have been found in others. When the outcome measures are not cognitive function or IQ, differences are less likely (Richardson et al. 1973; Graham and Adrianzen, 1979; Moodie et al. 1980; Bartel et al. 1978). There is very little information on children after school age (Ramalingaswami et al. 1993; Moodie et al. 1980) and it is impossible to conclude whether differences remain in adulthood. In

most follow-up studies, severely malnourished children have returned to poor environments where the standards of nutrition remain questionable. The behavioural abnormalities described in some of the studies e.g. (Galler et al. 1983b; Richardson et al. 1972b) may be partly explained by continuing poor nutrition, which could impede a child's full recovery.

Most investigators have opted to look at global IQ instead of examining a comprehensive range of cognitive functions. Perceptual-spatial functions and reasoning have been shown to be consistently affected (Nwuga, 1977; Hoorweg, 1976; Champakam et al. 1968). Although specific deficits in cognitive function may exist, data are insufficient to identify them at this stage. Several studies have found that motor skills are affected by malnutrition (Hoorweg, 1976; Cravioto and Arrieta, 1986; Galler et al. 1987) with children described as clumsy (Richardson et al. 1972), although not always (Bartel et al. 1978). Intersensory integration (Cravioto and Arrieta, 1986; Pereira et al. 1979; Champakam et al. 1968) and the aquisition of Piagetian milestones (Galler et al. 1987) are delayed in younger children.

School performance and behaviour.

Although most investigators have focused on malnourished children's cognitive development, a few have studied the behaviour of malnourished children. The focus of these studies has usually been children who have experienced an acute episode of severe malnutrition. Even when subjects and controls are matched for their school class, previously malnourished children have often been found to have poorer school grades than matched controls (Richardson et al. 1973; Galler et al. 1983; Galler et al. 1990). When subjects are matched with siblings, however, differences have not usually been found (Richardson et al. 1973; Graham and Adrianzen, 1979; Moodie et al. 1980) except in one case (Pereira et al. 1979). It

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may be that family attitudes towards education, poor attendance and late enrollment swamp any additional intellectual disadvantages from which the malnourished group may suffer.

Based on reports by mothers or teachers, formerly severely malnourished children have been reported to make poorer relationships with their peers and teachers, have poorer attention and are more easily distracted than their classmates (Richardson et al. 1972; Richardson et al. 1975; Galler et al. 1983). In addition, they have sometimes been reported to have less emotional control (Galler et al. 1983b) and be less active and more obedient (Hoorweg, 1976). Some of the inconsistencies in these findings can be explained by cultural variations in child rearing practices.

Problems with case-control design

A problem with most case-control studies is that there was no control for current dietary intake and nutritional status, or for the possible effects of hospitalisation, which may have contributed to the differences between the groups. There is also the problem with retrospective studies that the children may have been different before the onset of severe PEM, which means it is impossible to attribute equivalent development before the onset of malnutrition. In only one study (Cravioto and DeLicardie, 1972) were children examined from birth before the onset of severe malnutrition.

Conclusions about case-control studies

Despite the shortcomings of case-control studies, the findings of persistent deficits in cognitive function in malnourished children have been reasonably consistent across studies in developing countries where children returned to their homes. Using epidemiological principles (Hill, 1965), it is reasonable to attribute a causal relationship to early childhood malnutrition, and subsequent poor cognitive

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development, when children continue to live in deprived environments. However, marked improvements are possible with interventions.

Studies evaluating response to an improved environment

There are few reports of intervention programs designed to improve the development of severely malnourished children. Short-term programs of psycho-social stimulation during a child's stay in hospital have resulted in only transient improvements (McLaren et al. 1973; Cravioto and Arrieta, 1979).

In Jamaica, a long term intervention study of psycho-social stimulation was conducted with severely malnourished children (Grantham McGregor et al. 1994). It was fairly low cost and comprised visiting the children's homes weekly or two-weekly for three years. The intervened group showed rapid improvements in development compared with a non-intervened group, and the benefits were sustained for at least 16 years. A few studies have shown marked improvements in children who are adopted into vastly improved environments (Winick et al. 1975; Monckeberg, 1972; Colombo et al. 1992).

Low birth weight

Almost 25 million low birth weight (LBW) (<2500 g) infants are born each year, 95% of them in developing countries (World Health Organisation, 1984). In 1990-97, 10% of Jamaican infants had low birthweight. In developing countries, LBW infants are more likely to be born at term than those in developed countries, and are small due to intrauterine growth retardation (IUGR), secondary to maternal undernutrition and infection (Villar and Belizan, 1982). These babies are small for gestational age (SGA), meaning they were stunted in utero, and represent a major public health problem.

A recent review of 80 studies, mostly in developed countries, showed that LBW children generally have poorer levels of development than normal birthweight infants (Aylward et al. 1989). Fewer studies exist of SGA infants, and the findings have been inconsistent. One reason for this inconsistency is that there are no standard definitions of SGA, and there is no generally accepted cut-off point for SGA, although the WHO recommends using the 10th percentile for gestational age (de Onis and Habicht, 1996). In addition, SGA babies have a greater incidence of perinatal complications than NBW (normal birth weight) babies (Scott and Usher, 1966; Kramer et al. 1990), that may detrimentally affect their subsequent development (Low et al. 1985); children with perinatal complications have been inconsistently omitted from studies. Samples of SGA children generally have a large percentage of dropouts (Westwood et al. 1983; Pryor, 1992) that is often biased (Wariyar and Richards, 1989), with the least developed babies the most difficult to follow-up. Furthermore, most studies have been undertaken in developed countries, and findings in developing countries, where the children are exposed to more deprived environments, may be very different.

Studies of children in the first two years

Most studies evaluating children before 12 months, who had been SGA, have failed to find differences in developmental levels between SGA and NBW infants (Parmelee and Schulte, 1970; Low et al. 1978; Villar et al. 1984; Rubin et al. 1996). However, a recent Brazilian study showed that SGA babies scored significantly lower on both the Bayley mental and motor subscales at 6 months, and the difference increased by 12 months (Grantham-McGregor et al. pers. comm.) The development of SGA infants was affected by the quality of home stimulation and maternal literacy

at 6 and 12 months, and the development of NBW infants was not, indicating an increased environmental vulnerability in SGA children.

Four studies have compared SGA and NBW children between 12 and 24 months, and have found deficits in the mental development and behaviour of SGA groups (Low et al. 1978; Villar et al. 1984; Tenovuo et al. 1988; Nelson et al. 1996), but in most cases the differences were attributed to particularly vulnerable subgroups, such as those with congenital anomalies or very low birthweight.

Studies of children from two to seven years

The results of studies evaluating children from 2-7 years old indicate that children who were SGA performed significantly worse than children who were NBW in tests of cognition (Villar et al. 1984; Gorman and Pollitt, 1992), IQ (Babson and Kangas, 1969; Fitzhardinge and Steven, 1972; Pryor, 1992), language (Walther and Ramaekers, 1982), and school achievement (Hadders-Algra et al. 1988). In a few cases, the differences did not attain statistical significance (Babson and Kangas, 1969; Fitzhardinge and Steven, 1972), due to small sample sizes.

Some of the studies that evaluated children in the first two years of life continued to follow the children into later childhood. The American children evaluated by Rubin and colleagues (1988) were assessed at 4, 5, and 7 years old, and IQ, language development and reading readiness were worse in LBW children when controlling for gestational age, even without having shown differences at 8 months old (Rubin et al. 1996). Similarly, SGA Guatemalan children, who had not been different from NBW children before 12 months had significantly poorer developmental levels in their second year, and had lower scores on a cognitive battery verbal factor score than the NBW group at three years old (Villar et al. 1984; Gorman and Pollitt, 1992). Differences between the groups disappeared on

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subsequent evaluations at 4 and 5 years (Gorman and Pollitt, 1992). In one study (Fancourt et al. 1976; Harvey et al. 1992), the deficits in SGA children were entirely due to children who had shown growth retardation early in intrauterine life before the 26th week.

Studies of adolescents

Hack's comprehensive review of eleven studies on the effects of birth weight later in life (Hack, 1998), all from developed countries, indicates that adolescents, aged 10-19, who were SGA have lower mean IQs than adolescents who were NBW c.f. (Drillien et al. 1980; Paz et al. 1995), although the differences are significant in only some of the studies. Higher rates of mental retardation, subnormal school performance, learning deficits, and neurological abnormalities were also reported in adolescents who were SGA. However, one study from developing countries found no long term effects of SGA during adolescence (Pollitt et al. 1991).

Breastfeeding and fatty acids

According to UNICEF figures for 1990-1997, 44% of children in developing countries are exclusively breastfed from 0-3 months, 48% are breastfed with complementary foods at 6-9 months, and 51% are still breastfeeding at 20-23 months (State of the World's Children, 1999). No data are available for breastfeeding prevalence in Jamaica, but the mean for Latin American and the Caribbean is 39% exclusively breastfeeding from 0-3 months. In Nepal, 83% of infants are breastfed from 0-3 months.

Concurrent effects of breastfeeding

Eight studies have assessed the concurrent effects of breastfeeding on development in children 24 months old and younger (Morley et al. 1988; Temboury et al. 1994; Florey et al. 1995; Young et al. 1982; Ounsted et al. 1988; Morrow

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Tlucak et al. 1988; Rogan and Gladen, 1993; Morris et al. 1998) and show that breastfed infants appear to have a small but consistent advantage over non-breastfed infants in mental development, at all time points up to 24 months. The effects are more consistently statistically significant at the later ages, though small birth weight babies may benefit from as early as six months.

Longitudinal effects of breastfeeding

In most studies, the benefit of breastfeeding remained significant over time with the inclusion of socio-economic factors as covariants (Rodgers, 1978; Fergusson et al. 1982; Taylor and Wadsworth, 1984; Ounsted et al. 1984; Lucas et al. 1992; Rogan and Gladen, 1993; Niemela and Jarvenpaa, 1996; Horwood and Fergusson, 1998). However, some studies lost the significance of the findings (Jacobson and Jacobson, 1992; Gale and Martyn, 1996; Malloy and Berendes, 1998) and some did not include any control for social factors in the analyses (Hoefer and Hardy, 1929; Broad, 1979; Pollock, 1994).

Fatty acids

The brain is composed largely of lipids, a large proportion of which are long chain polyunsaturated fatty acids (PUFAs) which are critical because of their role in membrane phospholipid composition (Innis, 1993).

Animal studies have clearly shown that EFA deficiency results in deleterious and permanent brain damage (Crawford, 1993). There is some suggestion that EFA deficiency can also result in increased emotional reactivity (Wainwright et al. 1994). In humans, the effects of EFA deficiency are most evident in at-risk populations, particularly very-low-birth-weight babies, who are more likely to have AA and DHA deficiencies at birth (Carlson and Salem Jr. 1991; Crawford, 1993; Morley, 1996).

In general, EFA concentration in formula is insufficient for infants (Koletzko et al. 1987; Farquharson et al. 1992), as evidenced by studies showing that infants born pre-term who are fed EFA-supplemented formula have better visual acuity (Birch et al. 1992; Uauy et al. 1992; Makrides et al. 1993) and higher plasma levels of EFA than infants fed unsupplemented formula. However, the minimal data comparing the developmental outcome of pre-term infants supplemented with EFA suggest no clear results (Janowsky et al. 1995; Carlson and Salem Jr. 1991; Koletzko et al. 1987).

Growth

The effects of breastfeeding on linear growth are not consistent. In developing countries, increased breastfeeding has been associated with increased weight for age at 6-12 months of age (Hoefer and Hardy, 1929; Greiner and Latham, 1981; Taren and Chen, 1993; Adair et al. 1993; Cousens et al. 1993; Castillo et al. 1996; Gale and Martyn, 1996) and mid-upper arm circumference at 18 months (Briend et al. 1988). However, other studies have shown no benefit to growth in the first six months (Victora et al. 1998), and increases in malnutrition related to breastfeeding during the second year (Victora et al. 1984; Jansen, 1985; Brakohiapa et al. 1988; Victora et al. 1992), particularly in

the presence of other risk factors, such as low food intake and high diarrhoeal morbidity (Marquis et al. 1997). Based on an excellent Peruvian study examining the causality of the relationship between linear growth retardation and breastfeeding, it appears that poor growth and ill health leads to increased breastfeeding, rather than prolonged breastfeeding leading to malnutrition (Marquis et al. 1997).

<u>Immune response</u>

In both developing and developed countries, breastfeeding has been associated with an increased infant immune response (Pabst et al. 1997), resulting in decreased levels of gastrointestinal illness (Howie et al. 1990), respiratory tract and ear infection (Cunningham, 1979; Pullan et al. 1980; Victora et al. 1987; Scariati et al. 1997; Duffy et al. 1997), diarrhoea (Cunningham, 1979; Victora et al. 1987; Fergusson et al. 1989; Brown et al. 1989; VanDerslice et al. 1994; Scariati et al. 1997; Clemens et al. 1997) particularly in the first year of life, and chronic constipation (Malloy and Berendes, 1998).

Iodine deficiency

Iodine deficiency is the world's most common endocrine disease (Dunn, 1996). According to the 1993 WHO report, 1.6 billion people live in areas of iodine deficiency and approximately 20 percent of them have goitre. The total goitre rate for children 6-11 years old in 1985-97 was 44% (State of the World's Children). Iodine deficiency is the most common preventable cause of mental deficits and is a major public health issue (Hetzel, 1994; Delange, 1995). "Iodine deficiency disorders" (IDD) (Hetzel, 1983; Hetzel et al. 1990) include a wide range of conditions which affect growth, development and health. Depending on the duration and severity of iodine deficiency, the consequences range from minor psychomotor or mental retardation to severe cretinism. Iodine deficiency disorders can also take their toll socio-economically, with lower work output, per capita income, and less productive farm animals in iodine deficient areas (Dunn, 1996). The following discussion will be divided into observational studies and treatment trials.

Observational studies

Studies of goitrous and non-goitrous children

Studies evaluating goitrous children have shown inconsistent differences between goitrous children and non-goitrous controls in terms of outcome measures of intelligence (Rani et al. 1983; Muzzo et al. 1986; Escobar del Ray et al. 1989; Azizi et al. 1995). It may be that the results are not consistent because there is no clear relationship between level of hypothyroidism and the presence of goitre (Filteau et al. 1994).

Studies comparing children in iodine deficient and sufficient areas

In studies looking at the intelligence quotients of children living in iodine deficient villages as compared with children in iodine-sufficient areas, consistent results are obtained. Children in areas of iodine sufficiency have significantly higher levels of mental development when assessed with the WISC, Griffiths, or Bender-Gestalt test (Querido et al. 1978; Mehta et al. 1987; Bleichrodt et al. 1987; Boyages et al. 1989; Vermiglio et al. 1990; Azizi et al. 1993; Tiwari et al. 1996). In a meta-analysis of 18 studies evaluating the relationship between iodine levels in children and adults and cognitive function, individuals who had experienced some iodine deficiency had an IQ of 13.5 points lower than controls (Bleichrodt and Resing, 1994).

The observational studies discussed above paint a clear picture of iodine deficiency linked to poor mental development. However, most studies assessing the effects of iodine deficiency underestimate the complexity of factors that may affect iodine intake at the community or individual level. Many confounding factors may be present, such as socio-economic status, degree of isolation, access to health care, income levels, availability of water and electricity, quality of education, amount of

inbreeding, resource allocation, and other cultural issues, all of which may be responsible for the poorer developmental levels of the children.

Intervention studies

Maternal supplementation studies

Several studies have investigated the effects of iodized oil given to women before and during pregnancy on their children's mental development. Based on the extensive investigations of children from two Ecuadorian villages, for example, it is clear that iodination provides prophylaxis against severe developmental defects like cretinism (Ramirez et al. 1969; Ramirez et al. 1972; Trowbridge, 1972; Greene, 1994).

Using a more rigorous study design, Pretell et al. (Pretell et al. 1972) compared the children of mothers supplemented before conception from three iodine deficient villages, with unsupplemented controls from the same villages, and the children from treated mothers scored higher at all ages. In a double blind randomised trial in Zaire, the developmental quotients in infants from treated mothers were significantly higher than developmental quotients in infants from non-treated mothers (Thilly et al. 1980).

The best intervention study was conducted by Pharoah and Connolly in a longitudinal double blind randomised controlled trial in Papua New Guinea. Intramuscular iodised oil was effective in preventing both types of endemic cretinism (Pharoah et al. 1971), and children whose mothers received iodised oil had better cognitive and fine motor skills than control children (Pharoah et al. 1984). Infant and childhood cumulative mortality over first 15 years of life was significantly greater in control than in test group (Pharoah and Connolly, 1987).

The most recent maternal supplementation study, conducted in China, indicated that children born to women supplemented in the first and second trimester had decreased prevalence of moderate or severe neurologic abnormalities, decreased prevalence of microcephaly, and increased developmental quotients (Cao et al. 1994). All the studies discussed above indicate that maternal supplementation during gestation and the first trimester affects mental development in children. The studies from Papua New Guinea, China and Zaire are sufficiently robust to establish that iodine deficiency in utero causes cretinism and poor development in childhood.

Childhood supplementation studies

A limited number of researchers have conducted iodine supplementation trials in children, but many of the studies had design flaws. The earliest supplementation trials evaluating developmental outcomes with children took place in Ecuador (Dodge et al. 1969). The mean intelligence of the treated subjects was higher than that of the controls, but the results were only significant in the girls. In a Chinese study, researchers found that children from an iodine deficient area had significantly lower mean hearing thresholds, and the levels improved with treatment (Yan-You and Shu-Hua, 1985). However, there was no placebo group being measured at the same time.

In a double blind random controlled trial with school children in Bolivia, no improvements were reported in any of the neuro-developmental or IQ measures (Bautista et al. 1982). However, the iodine status of the placebo group improved during the study, confusing the results. A double blind placebo-controlled study in an area of endemic goitre was recently conducted in Malawi (Shrestha, 1994).

Significant differences were reported in the final test scores in three aggregate ratings on mental development comparing children who had received iodine with those who had received a placebo. However, there were no valid pre-test scores, so the findings are not conclusive.

Iron deficiency

The estimated prevalence of anaemia in children under five in developing countries is 46-51%, and in developed countries, 7-12% (DeMaeyer and Adiels-Tegman, 1985); 58% of pregnant women in developing countries have iron-deficiency anaemia. Iron deficiency alone affects approximately 2000 million people in developed and developing countries together, half of these with clinical iron deficiency anaemia and the other half with deficient body-iron stores. In adults, iron deficiency affects work capacity and work productivity (Scrimshaw, 1984).

Observational studies

Iron-deficiency anaemia and concurrent development

Many correlational studies have linked iron status with current development. For example, iron deficiency anaemia in poor, African-American 12-14 year olds was associated with lower achievement scores, lower scores on a visual after-image tests, and more behavioural problems (Webb and Oski, 1973; Webb and Oski, 1974). Similarly, a study of Asian 21-23 month olds living in the UK found that lower haemoglobin levels were associated with significantly lower scores on a developmental screening test of fine motor and social development (Grindulis et al. 1986); a study of Chilean children found associations between iron intake and educational achievement (Ivanovic et al. 1991); a study of children in the Philippines found associations between haemoglobin level and language achievement (Popkin and Lim-Ybanez, 1982). These associations between iron-deficiency anaemia and

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poor concurrent development are strengthened by pre-intervention measures of infants who were supplemented with iron. Iron-deficient anaemic infants scored lower on the Bayley test of mental development (Lozoff et al. 1982; Walter, 1983; Lozoff et al. 1987; Idjradinata and Pollitt, 1993) and on the Bayley test of motor development (Lozoff et al. 1982; Lozoff et al. 1987; Walter et al. 1989; Idjradinata and Pollitt, 1993). Only a few studies have failed to find associations (c.f. Johnson and McGowan, 1983).

Iron-deficiency anaemia and longitudinal development

Studies from both poor (de Andraca et al. 1990; Lozoff et al. 1991) and middle-class families (Palti et al. 1983; Palti et al. 1985) indicate that iron deficiency anaemia in infancy is associated with poor cognitive function in later in childhood. Non-anaemic Costa Rican five-year olds, who were anaemic at 12-23 months, scored less than control children on each of the outcome measures, which included tests of visual motor integration, picture vocabulary, motor proficiency, spatial vocabulary, and verbal and performance IQ (Lozoff et al. 1991). Similarly, non-anaemic Chilean five-year olds, who were anaemic some time between birth and 12 months, scored worse on a test battery including fine motor proficiency, psycho-linguistic ability and the pre-school abilities (Walter et al. 1989; de Andraca et al. 1990). In a similarly designed study, previously anaemic middle-class Israeli five-year olds scored lower than control children on the Wechsler IQ test (Palti et al. 1983; Palti et al. 1985). All of these studies attempted to control statistically for social factors that could contribute to the differences between groups, such as birthweight, home stimulation, and maternal IQ, education and depression. It could still be possible that differences in home environments accounted for the differences in scores of the two groups, therefore a causal relationship cannot be unequivocally

established. However, this correlational evidence indicates that iron-deficiency anaemia in infancy is associated with poor cognitive function in later childhood.

Treatment studies

Intervention studies with children under 2 years old

Studies of iron supplementation have shown no convincing or consistent evidence of benefit to children's developmental levels from short-term treatment of less than four weeks. Children who have received short-term treatment showed improvements in scores on the Bayley test of mental development in anaemic Chilean (Walter, 1983), and American children (Oski et al. 1983), although there were no placebo anaemic group in either study so that test practice could account for the improvement. No effects were found after treating anaemic American (Oski and Honig, 1978), Guatemalan (Lozoff et al. 1982), Costa Rican (Lozoff et al. 1987), or Chilean children (Walter et al. 1989).

Studies with longer term supplementation, of 2-6 months have yielded inconclusive, though somewhat more positive results. Aukett and colleagues (Aukett et al. 1986) in England found no significant differences after two months of treatment on the Denver mental development test, however, more iron-treated than placebo-treated children achieved a normal rate of development. In a non-clinical treatment trial in which all anaemic children were treated, Lozoff and colleagues (Lozoff et al. 1987) showed sustained lower test scores in most of the anaemic infants, but those infants showing the best response to iron therapy also showed the most improved test scores. However, Walter and colleagues (Walter et al. 1989) found no improvement with supplementation. One of the few studies that was a randomised controlled treatment trial reported a dramatic overall improvement in both mental and motor test scores (Idjradinata and Pollitt, 1993).

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Intervention studies with children over 2 years old

Fairly consistent evidence exists from studies supplementing children over two years of age. Benefits to the children's performance on tests of cognitive function or school achievement were reported in India (Seshadri and Gopaldes, 1989), Indonesia (Soemantri et al. 1985; Soemantri, 1989; Soewondo et al. 1989), Egypt (Pollitt et al. 1985), and the USA (Pollitt et al. 1983). It is unclear why one well-designed study in Thailand failed to find an improvement in school achievement when anaemic children were given iron (Pollitt et al. 1989).

Conclusions about iron deficiency and development

There is a well established association between iron deficiency anaemia and poor developmental levels, school achievement, and altered behaviour. In addition, early childhood anaemia predicts later poor development. Older anaemic children benefit from iron treatment in terms of cognition and school achievement, indicating that there is likely to be a causal association between iron status and mental development. In children under two years, however, the evidence is less consistent. Several studies have failed to show an improvement with treatment, although most have not been randomised trials. One well designed study in younger children showed a benefit, but the findings need to be replicated.

Other nutritional deficiencies

Vitamin A Deficiency

Vitamin A deficiency is associated with blindness and increased severity of infections such as measles and diarrhoeal disease. The 1995 estimates from the WHO Micronutrient Deficiency Information System database indicate that approximately 2.8 million children under five years old currently exhibit signs of clinical xerophthalmia, and 14 million pre-school children already have some eye

damage from vitamin A deficiency. Blindness would almost certainly have a detrimental effect on children's development and behaviour, especially where facilities for blind children are lacking.

Zinc Deficiency

Zinc deficiency has a questionable impact on mental development. Only two studies have examined the effects of zinc supplementation on cognitive development in children. These studies were carried out in 5-8 year old children in Guatemala (Cavan et al. 1993) and Canada (Gibson et al. 1989), and neither one found significant effects of treatment on a limited number of cognitive tests. In a recent Indian study, children who received zinc supplementation were found to be more active than a control group (Sazawal et al. 1996).

Short term hunger

Short-term hunger, which could be considered a stressor, has a concurrent effect of transient reduction in cognitive function, which is exacerbated in undernourished children (Simeon and Grantham-McGregor, 1989). Missing breakfast may negatively modify a child's behaviour, depending on the classroom environment. Providing breakfast to children has been shown to improve attendance and reduce tardiness.

Chapter 3: Linear growth retardation

Introduction

Height-for-age reflects linear growth that has occurred both pre- and post-natally. A child could have a low height-for-age due to normal variation in growth, or due to a deficit in growth. Stunting, linear growth retardation referring to shortness that is not genetic, occurs when a child has failed to reach genetic potential due to conditions of poor health or nutrition. The most widely used definition of stunting is height-for-age less than minus two standard deviations of the median of the reference population (Hamill et al. 1977).

Clearly, many factors contribute to the physical, social and emotional "well-being" of a child (Worthman and Panter-Brick, 1996). Thus, in assessing true well-being, children should be assessed along many dimensions in addition to anthropometry. Stunting (low height for age) is considered a much better cumulative measure of well-being for populations of children than underweight (low weight for age), because underweight is affected by weight recovery, and also by some children being overweight. Weight-for-age is very commonly used as an indicator because it is easier to measure.

Incidence and prevalence

An estimated 38% of children < 5 y.o. in developing countries and up to 52% of children in this age category in South East Asia are stunted (UNICEF, State of the World's Children, 1999). Levels of stunting in Jamaica have been reported to be 6%, but are actually likely to be higher. The United Nations Administrative Committee on Coordination, Sub-Committee on Nutrition has recently compiled

data from 95 countries in order to estimate the prevalence of stunting (**Figure 1**) and

61 countries to estimate trends in stunting (**Figure 2**) (ACC/SCN, 1997).
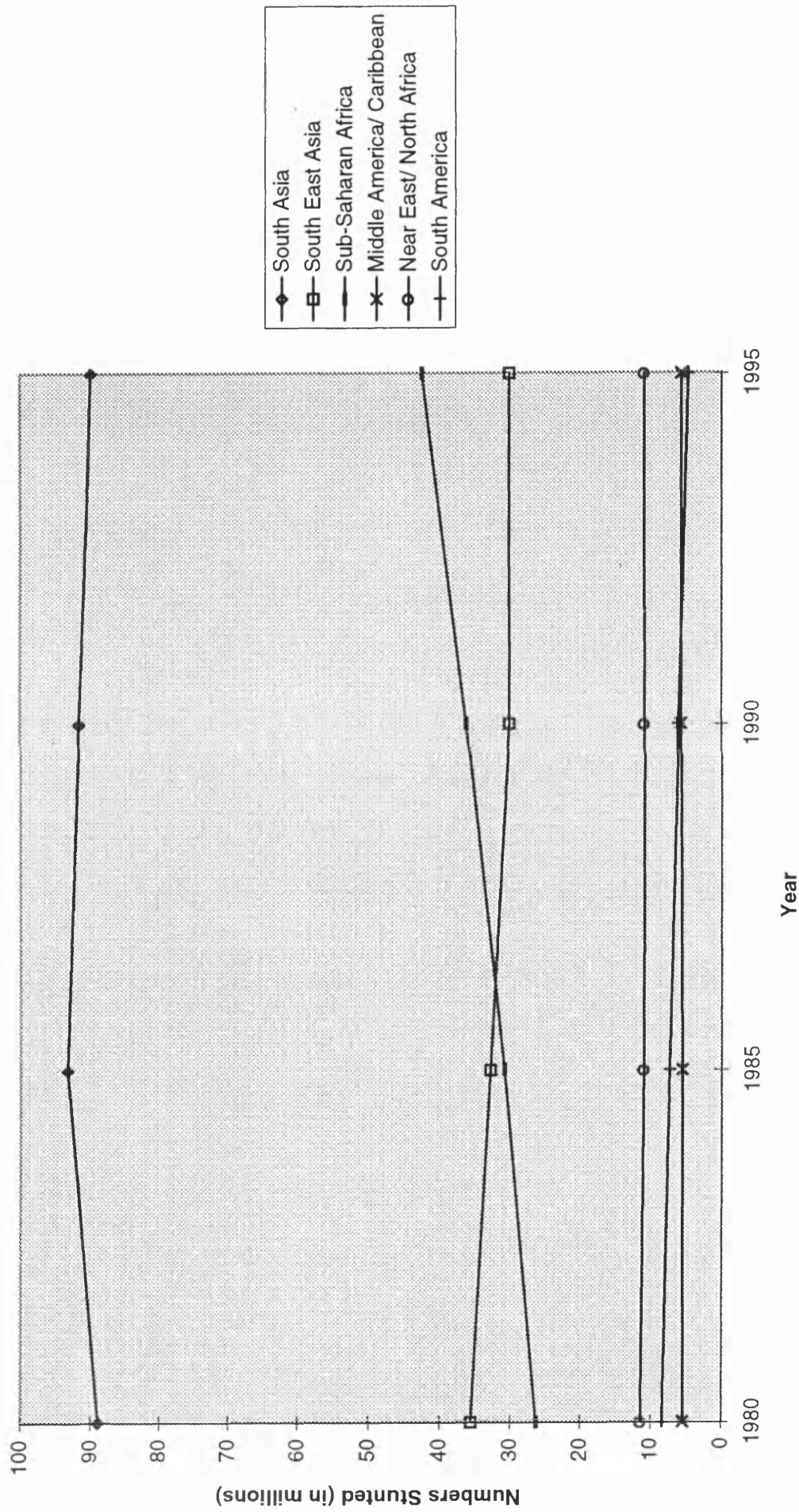
Figure 1: Estimated numbers of stunted children under five (in millions) for 1980, 1985, 1990 and 1995 by region (values from ACC/SCN 1997).
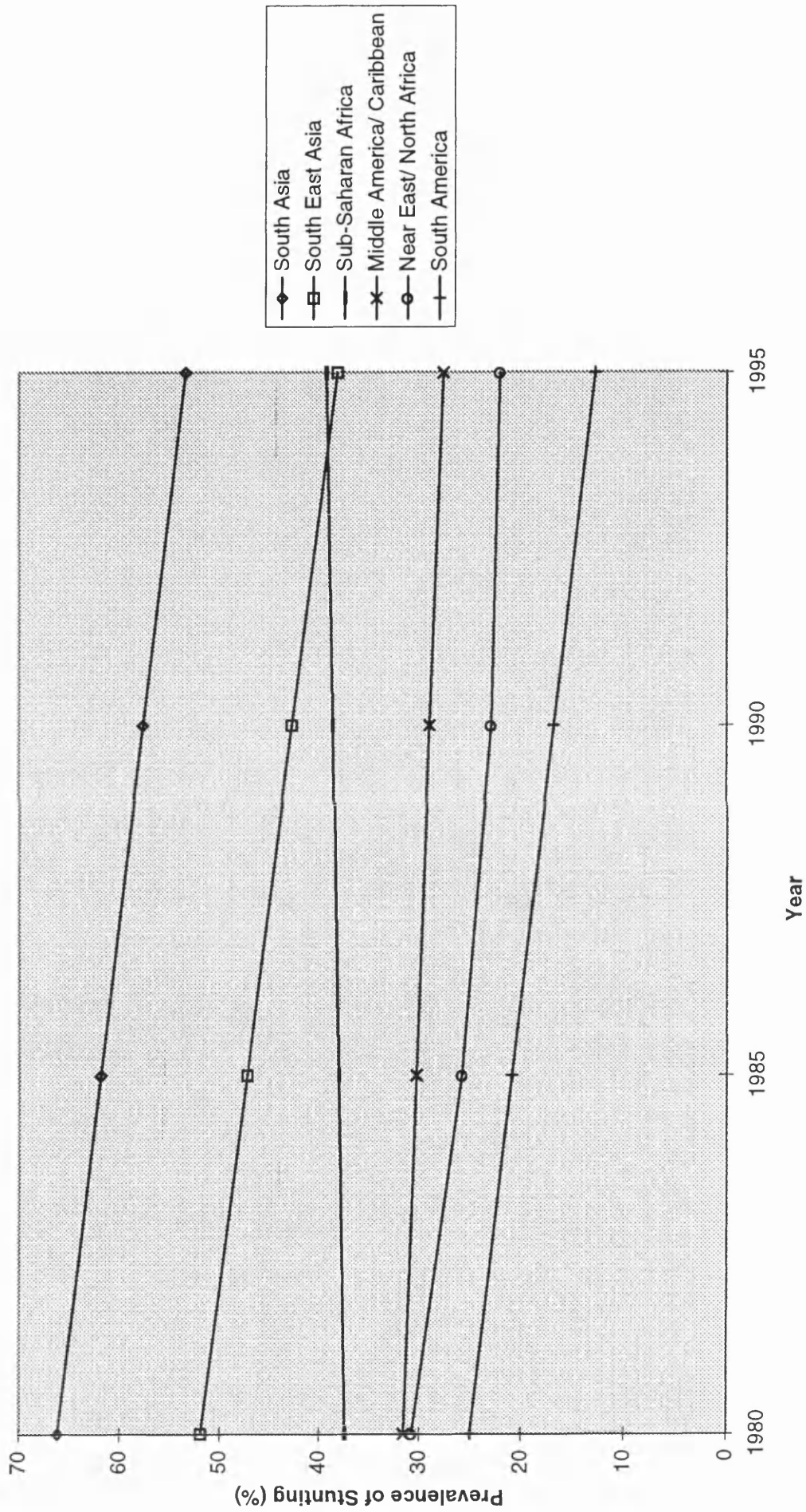
Figure 2: Estimated prevalence of stunting (%) in children under five for 1980, 1985, 1990 and 1995 by region (values from ACC/SCN 1997)

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The estimates for numbers of children stunted were calculated using prevalence values of stunting in children less than five years old, and 1996 population estimates from the United Nations. In this way, the estimates were calculated to include all countries in each region, even those for which there was no survey to contribute to the prevalence estimation. From 1980 to 1995, the combined number of stunted children in the six regions increased by 5%, from 175.8 to 183.9 million. Stunted Chinese children have not been included in the calculations because only one survey from this region exists. Values from the survey in 1992 show 36.1 million children in China who are stunted.

The trends for each country were calculated using a linear regression model assuming a straight-line relationship of stunting over time. Only one region showed a non-linear relationship (Near East/ North Africa), and thus, a quadratic model was used to approximate the non-linear relationship. Country population was included in all models so that the larger countries in each region would contribute more to the estimates for that region than the smaller countries.

Overall, during the 15-year period 1980-1985, the prevalence of stunting decreased globally by approximately 0.54 percentage points per year. In five of the six regions, the trends were negative and statistically significant, ranging from a decrease in percentage of children stunted of 0.26 and 0.64 in Middle America/ Caribbean and Near East/ North Africa, to 0.80, 0.84 and 0.90 in South America, South Asia and South East Asia, respectively. The trend in Sub-Saharan Africa showed an increase in average prevalence of 0.13 percentage points per year.

Causes of linear growth retardation

Height reflects the genetic potential of a population, as well as the socio-economic, health and nutritional conditions affecting the population (Martorell et al. 1988). Linear growth retardation is multifactorial, but has been explained by three major causal factors: poor nutrition, high levels of infection, and problematic mother-infant interaction, which is closely related to the socio-economic status of the family (Waterlow, 1994a).

Nutrition and infection

In developing countries, poor nutrition plays a major role in the aetiology of stunting, though no specific nutrient has clearly and consistently been linked with growth retardation. Energy, protein, zinc and iron deficiencies have all been implicated, (Allen, 1994), and the data are strongest about zinc (Prentice and Bates, 1994), protein (Lampl and Johnston, 1978; Golden, 1988) and fats from animal foods (Dagnelie et al. 1994). To a lesser extent, frequent or prolonged infections, particularly those causing diarrhoea, also play a role (Allen, 1994).

Environmental influences

The role of the environment is clearly illustrated by the secular improvements in average height shown by populations over the last century in both European and non-European countries (Lynn, 1989; Ulijaszek, 1994). For example, in 1833, British children were as tall as children today who are from poor countries such as Indian and Guatemala (Martorell et al. 1988). The growth curve (height against age) of Jamaican primary schoolchildren (7.5 - 14.5 years old) in 1964 fell almost halfway between the curve for English (London) children in 1905-1912 and English children (London) in 1959 (Ashcroft and Lovell, 1966).

Although the impact of genetic influence of growth cannot be denied (Davies, 1988), the environmental effects of poverty generally outweigh the effects of genetic potential, particularly in regions where economic development is slow. When the level of socio-economic development rises, poverty becomes less of a limiting factor, and genetic variations play out much more strongly (Martorell et al. 1988).

The causes of stunting are likely to vary according to several environmental factors. Pregnancy weight gain, lactation, diet quality, supplemental feeding use, diarrhoeal disease, parental size, household sanitation and other cultural practices have been shown to have differential effects on stunting prevalence depending on the cultural context (Neumann and Harrison, 1994).

Timing of stunting

Infancy and childhood

The age of onset of stunting varies, but usually occurs in the first two to three years of life (Keller, 1988; Waterlow, 1988). The "nutrition-dependent" infancy phase of growth begins mid-gestation, and continues, with decreasing influence until a child is 3-4 years old (Karlberg et al. 1994). At 6-12 months old, the "growth-hormone dependent" childhood phase begins, and continues in addition to the infancy phase. Growth faltering has been shown to be related to delayed onset of the childhood phase of growth, with the age of onset a key discriminator between normally growing infants and infants showing early growth faltering (Karlberg et al. 1994). Throughout the first 24 months of life, growth is discontinuous and saltatory, with short bursts of growth punctuating long periods of stasis (Lampl et al. 1992).

These data suggest that stunting could result either from decreased frequency or amplitude of growth spurts.

In the first few months of life, infants in developing countries grow just as quickly as children used in reference populations from developed countries. Growth retardation starts from the second to the sixth month of life, and in developing countries is most often associated with weaning (Martorell, 1989). Infants are at particular risk during this time because of their high levels of nutritional requirements and concurrently high rates of infections. Infants who are exclusively breastfed are protected against early post-natal stunting (ACC/SCN, 1997).

Long-term anthropometric implications

Almost complete catch-up in growth can be achieved when children experience dramatic environmental change and an improved diet, such as occurs during adoption (Golden, 1994). This phenomenon is very unusual in the context of the developing world where poor conditions prevail (Martorell et al. 1994; Golden, 1994). Low nutritional status is associated with several additional environmental factors such as poor housing, and low maternal literacy, which make catch-up growth even less likely (Powell and Grantham McGregor, 1985). In poor communities, even long-term nutritional supplementation of stunted children is unlikely to result in complete catch-up growth (Costello, 1989; Walker et al. 1996). There is usually a strong relationship between pre-school height-for-age and adolescent height (Simondon et al. 1998).

Studies of the relationship between height and development

Introduction

Some researchers have argued that stunted children are "small but healthy," that stunting has no functional implications because of the minimal contribution of poor nutrition and infection, and that the real risks of malnutrition to health occur only with wasting (Seckler, 1980). However, this theory is largely unfounded because nutrition and infection are highly implicated in all known mechanisms whereby poverty affects growth. Nutrient intake and infection, both of which directly affect nutrient availability, are influenced directly by food resources (contingent on land and income), infant feeding practices, health practices and environmental sanitation, all of which are influenced by socio-economic status (i.e. wealth, education, etc.) (Martorell, 1989).

Study design limitations

Stunting in poor populations is usually associated with poor mental development. However, the many socio-cultural and economic disadvantages that coexist with stunting (Martorell et al. 1988) may also detrimentally affect mental development, making it difficult to determine whether the poor development of stunted children is due to nutritional deficiency or whether stunting is just an indicator of poverty. Thus, it is important to control for social background as much as possible in study design and statistical analysis.

Study types

To understand the association between height and mental development in children, investigators have taken a variety of approaches. Some have examined cross-sectional correlations, and have either disregarded or controlled for the children's socio-economic backgrounds, with varying degrees of rigour. Others have

looked for longitudinal associations between change in height and change in mental development, or have examined associations between height in early childhood and later mental development. Some other investigators have conducted nutritional supplementation studies, which are probably the way to determine whether the link between stunting and poor mental development is related to nutritional factors.

Cross sectional Associations

Height for age and cognitive development

Most cross-sectional studies have found significant associations between height-for-age and children's cognitive development (Cravioto et al. 1966; Chun, 1971; Monckeberg, 1972; Powell and Grantham McGregor, 1980; Freeman et al. 1980; Bogin and MacVean, 1983; Moock and Leslie, 1986; Jamison, 1986; Agarwal et al. 1987; Florencio, 1988; Sigman et al. 1989; Clarke et al. 1991; Paine et al. 1992; Huda, 1997), although a few have not found any significant associations (Church and Katigbak, 1991; Colombo et al. 1988; Wachs et al. 1992) for inconsistent reasons. The associations between height for age and cognitive function or school achievement in school-aged children are shown in **Table 1**.

Table 1: Associations between height for age and cognitive function or school achievement in school-aged children

Study	Sample[1]	Measurements	Results
Positive findings			
Huda pers. comm., Bangladesh	N=340 6-9 y.o.	Height for age School Achievement Cognitive battery (Raven's matrices, fine motor tasks, information processing)	Height for age associated with math score (P=0.0006), learning (P=0.013), and fine motor tasks (P<0.01) when controlling for SES
Paine et al. 1992, Brazil	N=226 7-10 y.o.	Height for age Cognitive assessment (Goodenough-Harris drawing test, Non-verbal intelligence test, Raven's matrices, WISC-R coding and block design subtests)	Height for age associated with WISC coding (r=0.41, P<0.01) and WISC block design (r=0.32, P<0.05) with no control for SES
Clarke et al. 1991, Jamaica	N=755 9.5-12.5 y.o.	Height for age School achievement (WRAT)	Height for age predicted school achievement controlling for SES
Sigman et al. 1989, Kenya	N=133 (60 girls, 73 boys) 7-8.5 y.o.	Height for age Cognitive assessment (Peabody Picture Vocabulary Test, Raven's Progressive Matrices)	Girls: significant correlation between height for age and cognitive scores (r=0.35, P<0.05), remain with SES, Boys: non-significant correlation between height for age and cognitive scores (r=0.21).
Florencio 1988, Phillippines	N=2304 7-12 y.o.	Height for age School achievement tests	Significant correlation (r=0.14, P<0.0001). Significance remains with control for SES

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Study	N / age	Variables	Results
Agarwal et al. 1987, India	N=1336 6-8 y.o.	Height for age Mental ability (intellectual ability, perception, memory and reasoning)	Children <90% height for age lower scores on tests of mental ability (97.7 v. 89.9) with control for SES
Jamison 1986, China	N=3149 7-15 y.o.	Height for age School grades behind where should be according to age	Significant correlation (P<0.05)
Moock & Leslie 1986, Nepal	N=350 5-11 y.o.	Height for age School grades ahead of age-appropriate grade	Height for age is significant (P<0.05) in regression predicting school achievement from several anthropometric and socio-economic variables
Bogin & MacVean 1983, Guatemala	N=144 children 7-11 y.o. (grades 1, 3, 4, and 5)	Height Intelligence level (Americano de Guatemala, Pinter Durost, or Otis, depending on grade level)	Girls: no significant correlations at any age (range r= -0.05-0.03) Boys: significant correlations (range r=0.15-0.27, P<0.05 for grades 3 and 5), significance remains with control for SES
Freeman et al. 1980, Guatemala	N=412 3-7 y.o. (same study as Lasky, listed below)	Height Mental assessment (language facility, short-term number memory, perceptual analysis)	Composite mental assessment score significantly correlated with height at all ages in both sexes (range r=0.15 to 0.33, P<0.01 for all) Significance remains when controlling for SES

Powell & Grantham-McGregor 1980, Jamaica	N=389 11-13 y.o.	Height for age School achievement (WRAT)	Height for age significantly (P≤0.01) predicted score on WRAT
Mönckeberg 1972, Chile	N=108 3-5 y.o.	Height for age General DQ	Positive correlation (r=0.41, P<0.001)
Chun 1971, Singapore	N=1123 male 12 y.o.	Height School achievement	Taller children more likely to pass Primary IV examination
Cravioto et al. 1966, Mexico	N=143 6-11 y.o.	Stunting Neuro-sensory integration	Association in poor rural children but not in middle class children
Negative findings			
Wachs et al. 1992 Egypt	N=110 7-10 y.o.	Height for age Cognitive assessment (Egyptian version of WISC-R, Raven's Colored Matrices)	No significant correlations
Church & Kagitbak 1991, Phillipines	N=177 5-6 y.o.	Height for age Cognitive battery (inc. visual recognition, perceptual reasoning, spatial organization, etc)	No significant correlations
Colombo et al. 1988, Chile	N=68 7-10 y.o.	Height for age IQ (WISC)	No significant correlations

[1] y.o. years old, m.o. months old, d.o. days old, SES socio-economic status, DQ developmental quotient, WISC Wechsler Intelligence Scale

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Associations between height-for-age and school achievement have been found in older children without controlling for socio-economic status (Chun, 1971; Powell and Grantham McGregor, 1980; Jamison, 1986; Paine et al. 1992). Even after controlling for socio-economic conditions, investigators have found significant associations between height for age and IQ, cognitive function or school achievement levels in school-aged children in many countries, including the Philippines (Florencio, 1988), Jamaica (Clarke et al. 1991; Grantham McGregor et al. 1997), Guatemala (Johnston et al. 1987), Nepal (Moock and Leslie, 1986), Kenya (Sigman et al. 1989b), Bangladesh (Huda, 1997), and India (Agarwal et al. 1987).

Significant associations have also been found between stunting and poor psychomotor development in young children (**Table 2**), in Guatemala (Lasky et al. 1981), Chile (Monckeberg, 1972), Jamaica (Powell and Grantham McGregor, 1985), and Kenya (Sigman et al. 1989a). Only a few studies have failed to find significant associations between height and measures of mental development or school achievement (Church and Katigbak, 1991; Bogin and MacVean, 1983; Wachs et al. 1992; Colombo et al. 1988). The reason for the negative findings is not always clear, but small samples (Colombo et al. 1988) may explain some findings.

In contrast to the findings with height-for age, weight-for-height has only been occasionally (Lasky et al. 1981; Sigman et al. 1989; Popkin and Lim-Ybanez, 1982) associated with children's development.

Table 2: Associations between height for age and cognitive and motor development in infants and pre-school children

Study	Sample	Measurements	Results
Positive findings			
Sigman et al. 1989a, Kenya	N=110 18-30 m.o.	Height Development (Bayley Motor and Mental Scales, home observation of play)	Bayley scores correlated positively with length (r=0.25, P<0.05), Simple play correlated negatively with length (r= -0.24, P<0.05), and more advanced functional and symbolic play correlated positively (r=0.05, r=0.13 respectively)
Powell & Grantham-McGregor 1985, Jamaica	N=168 6-30 m.o.	Height for age IQ (Griffiths Mental Development Scales, hand & eye coordination, hearing & speech, performance subscales.)	correlation (r=0.33, P<0.01) height for age predicts DQ (P<0.01, 33% variance explained) when controlling for child's age, sex, birth order, and family SES
Lasky et al. 1981, Guatemala	N=418 assessed at 6 m.o. Same infants assessed at 15 (N=383) and 24 m.o. (N=334)	Height Mental and motor assessment (Composite Infant Scale: items from Bayley, Gesell, Psyche Catell, Merrill-Palmer, Stanford-Binet modified for Guatemalan context)	Mental and motor performance at 6, 15 and 24 correlated with length at 6, 15 and 24 months (range, r=0.18 to 0.35, P<0.01 for all)
Mönckeberg 1972, Chile	N=118 1-3 y.o.	Height for age General DQ	1-3 y.o.: Positive correlation (r=0.56, P<0.001)
Negative findings			
Colombo et al. 1988, Chile	N=228 247-274 d.o. N=42 51-65 m.o.	Height for age DQ (Chilean scale) Height for age IQ (WISC)	No significant correlation No significant correlation

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Height for age and other functional associations

Stunting has also been associated with poor fine motor skills in children from Bangladesh (Huda, 1997), and Jamaica (Grantham McGregor et al. 1997), and poor neuro-sensory integration in children from Mexico (Cravioto et al. 1966). In the Mexican study, the association was present in poor rural children but not in middle class children. The authors suggested that stunting in middle class children was due mainly to genetic tendencies, and was thus not associated with functional deficits, whereas stunting in the poor rural children was attributed mainly to poor nutrition which was associated with consequent functional deficits.

Height for age and behavioural development

A small number of studies have looked at the behaviour of mild-moderately malnourished infants, and even fewer of mild-moderately malnourished school-aged children. However, the results are fairly consistent: unsupplemented young children have been described as more quiet, reserved, withdrawn, and timid, with great difficulty making up their minds, and a fear of novel situations (Chavez and Martinez, 1979). When they were older, the children spent less time on task in the classroom, and cried and slept more then the supplemented children. Underweight children have been shown to be more anxious, less imaginative in their problem-solving approaches, and less environmentally involved than children of normal weight (Barrett and Radke-Yarrow, 1985).

Stunted children between 9 and 24 months of age have been found to have behavioural differences including delayed motor development and consequent decreased activity levels (Meeks-Gardner and Grantham-McGregor, 1994). They have been shown to be less exploring and happy than non-stunted children (Meeks-Gardner et al. 1997).

There are also limited data on the activity of free-living, mild to moderately under-nourished children. Several investigators have reported that they are less active (Meeks Gardner et al. 1995; Torun, 1989; Rutishauser and Whitehead, 1972). However, very few investigators have studied the children's development as well as their behaviour. Chavez and Martinez (1982) showed that unsupplemented children were less active and had lower DQ's than supplemented children; however, the lower DQ's preceded the reduced activity levels (Grantham-McGregor et al. 1990).

Longitudinal Associations

In children who have recovered from marasmus or kwashiorkor, height-for-age in the acute stage is usually a better predictor of mental development than weight-for-height or oedema (Hoorweg, 1976; Grantham McGregor et al. 1982). In both Guatemala (Pollitt et al. 1993) and Jamaica (Powell et al. 1995), change in height over a two year period in the first three years of life was related to change in development.

Supplementation Studies

Preventative

Several preventative supplementation studies have been conducted in populations with endemic undernutrition. Although these studies were not planned specifically to examine stunting, the findings are relevant in cases when both the height and development of the children improved. In three early studies of preventive supplementation, in Guatemala (Freeman et al. 1980), Columbia (Waber et al. 1981), and Mexico (Chavez and Martinez, 1982), pregnant women were given nutritional supplements and then their offspring were supplemented for three or

more years. The supplemented children showed gains in height and cognition compared with non-supplemented children.

The only supplementation study aimed specifically at stunted children was conducted with stunted and non-stunted Jamaican children aged 9 to 24 months (Grantham McGregor et al. 1991). The stunted children received nutritional supplementation for two years with or without psychosocial stimulation. Supplementation and stimulation produced independent benefits to the children's mental and motor development. The benefits from a combination of supplementation and stimulation were additive, and only the children receiving both treatments caught up to the non-stunted control group in developmental levels. The implication of these findings is that at least part of the deficit in the development of stunted children is due to poor nutrition. However, both stimulation and supplementation are necessary to improve the development of stunted children to culturally appropriate levels.

Supplementation follow-up studies

When the Latin American children were followed-up some years later, benefits remained to cognition, and school achievement (Chavez et al. 1994; Pollitt et al. 1993), and reading readiness (Herrera and Super, 1983). Follow up of the Jamaican children at seven years of age showed that only small global benefits from intervention remained (Grantham McGregor et al. 1997). However, even the control children showed marked catch-up in height, reflecting the improving nutritional status of the Jamaican population. In the Guatemalan study (Freeman et al. 1980) benefits were not apparent at six years but were present at adolescence (Pollitt et al.

1993), suggesting that larger differences between groups in the Jamaican study may reappear at a later age.

Vulnerable age

Evidence for a vulnerable period when nutrition is particularly important for mental development is not conclusive. In one supplementation study, children receiving supplements after 24 months of age showed fewer and smaller benefits than children receiving supplements earlier (Pollitt et al. 1993). However there were fewer children receiving supplements after 24 months. In a study with undernourished children in Cali, Columbia (McKay et al. 1978) an integrated program of stimulation and supplementation was given to children for different periods of time, beginning at different ages. Those children receiving supplementation from the youngest age (42 months) showed the greatest and most sustained benefits. However it was not possible to separate supplementation from stimulation, and duration was confounded with age.

Four studies showed that height in early childhood predicted cognitive development at a later age. In a Guatemalan study (Martorell et al. 1992) the children's height at 3 years of age predicted their performance on tests of numeracy, literacy, general knowledge and adolescent school grade attainment. Similarly, in a Kenyan study (Sigman et al. 1991), height in children between 18 and 30 months of age predicted cognition scores at five years of age, and in a Jamaican study (Richardson, 1979) height at one year of age predicted school achievement scores between 9 and 11 years of age in girls, but not boys. In a recent study of stunted Jamaican children, height-for-age between 9 and 24 months of age predicted IQ at 7 years of age better than concurrent height (Grantham McGregor et al. 1997). These

findings suggest that nutrition in the first two to three years is critically important to children's development.

Mechanisms

The precise mechanism linking stunting to poor mental and behavioural development is unknown. Given the environmental and nutritional associations with stunting, it is possible that the mechanism varies according to which nutrients are deficient, or that several hypothesised mechanisms which could act separately or together.

One early hypothesis is that undernutrition directly affects central nervous system activity, which subsequently affects mental and behavioural development and functioning (Stoch and Smythe, 1967).

Another accepted possibility is that of functional isolation, wherein activity level is the mediator between poor nutrition and poor development (Levitsky, 1979). According to this theory, children who have experienced undernutrition are smaller and less active than their well nourished peers. Another possible mechanism is that the children's small size could lead adults to treat them like younger children and not provide age appropriate stimulation. As a consequence, the undernourished children are treated as if they were younger, and are therefore functionally isolated from environmental stimuli that could potentially enhance their development. However, this hypothesis has not been supported by recent research investigating activity levels in stunted children, which found that early differences in infantile activity level were not sustained after six months. (Meeks Gardner et al. 1995)

Similarly, undernutrition may cause apathy and subsequently reduced activity and exploration. This phenomenon has been described in iron (Lozoff et al. 1985),

zinc (Bentley et al. 1997; Sazawal et al. 1996) and energy deficiency (Viteri and Torun, 1981; Torun and Viteri, 1981).

Conclusions about linear growth retardation

Stunted children have multiple functional disadvantages and fail to reach their potential in mental development and school achievement. Poor development and educational levels in stunted children are likely to limit personal and national productivity (Schiefelbein, 1983; Lewin et al. 1982). Stunted adults may also not be able to promote good development and health in their children (Caldwell, 1979; Wagner, 1974; Levine, 1980) which in turn leads to an inter-generational effect. Poor nutrition almost certainly plays a role in the development of stunting, as well as poor stimulation and other components of the socio-cultural environment.

Chapter 4: Stress physiology and related behaviours

Introduction

Clearly, children who are from developing countries must deal with numerous life stressors. They are more likely to live in nutritionally, psycho-socially, materially and economically deprived situations, all of which could contribute to enhanced stress levels.

In addition, interpersonal violence among youth is a critical problem in many countries in the Americas (Uva, 1990; Slaby and Stringham, 1994; DuRant et al. 1994). Violence has been acknowledged to be a major public health concern by the Pan American Health Organisation (Health and Violence: Regional Plan of Action, Washington D.C., PAHO/HPP/94.11) and the Jamaican Ministry of Health (Sectoral Debate 1996). By 1995, there were 67 homicides and shootings per 100,000 population in the Kingston-St. Andrew area, according to figures from the Statistics Division at Police Headquarters. Over half of the admissions for trauma at the University Hospital of the West Indies in 1992 were due to interpersonal violence (Crandon et al. 1994).

Corporal punishment by parents is common and generally accepted in Latin America and the Caribbean (Krugman et al. 1992; Anderson and Payne, 1994; Payne, 1989). For example, a retrospective Costa Rican study of 497 randomly selected University students reported that 80% of the subjects had been spanked as children, 30% had been whipped, and 0.5% had experienced very severe forms of punishment such as burning or tying to a tree. A recent study of 13-14 year old girls

from inner-city Kingston showed that two-thirds of the girls had been beaten during one school year (Walker et al. 1994).

Definition of "stressor"

Stressors have been defined as external or internal disturbing forces that challenge the equilibrium or homeostasis of an organism (Chrousos and Gold, 1992). These disturbances include physical stressors such as trauma, prolonged exposure to cold or heat, surgery, injection, prolonged heavy exercise, infection, pain, and decreased oxygen supply, and psychological stressors such as fear, high ego involvement, low predictability, low controllability, competition and novelty.

In 400 BC, Hippocrates emphasized that health necessitated "balance" and challenges to that balance resulted in disease. However, stress physiology did not emerge as an academic discipline until the beginning of this century, when Walter Cannon demonstrated the role of epinephrine in responsiveness and coined the term "flight or fight response." By the 1930's, Hans Selye had discovered that stressors provoked the secretion of glucocorticoids. He used the term "general adaptation syndrome" to refer to the stress response he observed in rats who developed enlarged adrenal glands and peptic ulcers when subjected to various unpleasant events, such as surgical injury, excessive exercise, injections, cold or illness (Selye, 1936).

It was clear to the first stress researchers that some situations of threatened homeostasis could be perceived as eustress, or positive simulation for performance and development. The cascade of hormones released in response to stressors can result in increased energy or cognitive acuity to facilitate escape, or decreased reproductive and immune response to save limited physical resources. However, the generally accepted concept of stress usually revolves around distress, meaning more

severe, protracted, uncontrollable, unpleasant circumstances, which result in the over-stimulation of the stress response system.

A fitting analogy would be of the Yerkes-Dodson law, which is the fundamental law linking arousal to performance. The law states that the quality of performance for any task is an inverted U-shaped function of arousal, with very low and very high levels of arousal associated with poor performance, and mid-level arousal necessary for peak performance (Kahneman, 1973). The range over which performance improves with increasing arousal varies with task complexity. Yerkes and Dodson formulated the law based on experiments with rodents showing that certain levels of shock intensity facilitated learning, but that much higher levels resulted in reductions in performance. A similar curve has also been more recently popularized and illustrates the relationship between physiological arousal and performance (Henry, 1992).

Adaptational responses to physical and psychological stressors are designed to preserve homeostasis, and release the body from a state of disharmony. In the most basic sense, the goal of relieving stress is satisfied by adaptive redirection of energy, oxygen and nutrients to the central nervous system and muscles. Behaviourally, this adaptation is manifest by increased arousal, alertness, and vigilance, and more focussed attention, as well as suppressed reproductive and feeding behaviour. Physical manifestations include increased respiratory rate, vascular activity, gluconeogenesis, lipolysis, blood pressure and heart rate, and decreased inflammatory or immune response.

All of these adaptations result in positive gains in the short run. For instance, when scared, an animal can escape more quickly without wasting energy on

reproduction or immune function. When competing, a gymnast has increased ability to focus her energies. However, all of these behavioural adaptations can be problematic when over-triggered. The stress response is perfect for short-term responsivity, but can result in stress-related disease when over-utilized.

Selye's early work identified the same "generalized adaptational response" to a whole variety of stressors. Though current research has shown that many facets of the stress response are generalized and non-specific, some adaptive responses are specific to the stressor, and the response is generally determined by the magnitude, not the direction, of the homeostatic imbalance (Sapolsky, 1993). In addition, it is becoming even more clear that specific perceptions of control, fear and vulnerability result in very different patterns of physiological activation and responsivity across the human population (Henry, 1992).

Physiology of the stress response

<u>Overview</u>

Behind these behavioural and physical responses to stressors is a complicated physiological response that can be divided into two major pathways: the hypothalamic-pituitary-adrenocortical (HPA) system and the sympathetic-adrenal-medullary system (Axelrod and Reisine, 1984; Chrousos and Gold, 1992; Sapolsky, 1993).

When the brain first perceives a stressor, neural input to the hypothalamus induces secretion of corticotropin releasing hormone (CRH), which is released in a pulsatile fashion. CRH is carried by the hypothalamo-pituitary portal vessels to the anterior pituitary. This action then stimulates adrenocorticotropic hormone (ACTH) release, which circulates to the adrenal cortex and stimulates glucocorticoid release.

Vasopressin, also released by the hypothalamus, acts synergistically to potentiate the effects of CRH on the cells of the anterior pituitary.

Glucocorticoids are steroid hormones, and the dominant form released in humans is cortisol (hydrocortisone), whereas the primary form released in rodents is corticosterone. Within a few seconds of perception of a stressor, CRH secretion increases. ACTH secretion follows about 15 seconds later, and glucocorticoid concentration increases within a few minutes (Sapolsky, 1993). Following exposure to a stressor, it typically takes 20-30 minutes for cortisol levels to reach their peak, and it may take several hours for the cortisol to be cleared from the plasma (De Kloet, 1991).

Three major pathways mediate the HPA stress response: direct stimulation of the hypothalamus and pituitary by biochemicals arriving in the general circulation, direct sensory signals travelling to the hypothalamus through brain-stem pathways, and psychological stimuli transmitted to the hypothalamus from the cerebral cortex via limbic circuits (De Kloet, 1991). The debate continues over how the HPA response is triggered and controlled (Sapolsky, 1993).

The second major pathway of the stress response involves the sympathetic-adrenal-medullary response. The locus ceruleus-norepinephrine/ sympathetic nervous system, located on the brain stem, is activated and releases norepinephrine, which results in increased vigilance, arousal and anxiety. Epinephrine is also released from the adrenal medulla. Brief psychological stressors have been shown to heighten both cardiac activation and plasma catecholamine concentrations (Cacioppo et al. 1995).

Adreno-cortical response

Cortisol: structure and function

Most cortisol that is released is bound by proteins, particularly corticosteroid-binding globulin (CBG). However, about ten percent of released cortisol is unbound and is therefore biologically active. A negative feedback mechanism exists whereby cortisol acts on receptors in the brain to inhibit further production of CRH and ACTH and thus reduce further cortisol release.

Cortisol has a circadian rhythm related to sleep-wake activity and is secreted in bursts that peak in the early morning (Wallace et al. 1991; Kirschbaum and Hellhammer, 1989). Alterations in the patterns of pulsatile secretion have been noted in endogenous depression, with depressed people having earlier timing of the first secretory episode. Variations in the diurnal pattern of cortisol secretion have also been noted in unemployed subjects, who had higher morning and lower evening levels when compared with employed subjects (Ockenfels et al. 1995).

Within the first half-hour after awakening, free cortisol levels show a 50-75% increase, with levels decreasing during the next half hour to return to baseline levels an hour after awakening. No associations have been found between early morning cortisol and sleep duration or time of awakening, although the timing of sample collection relative to time of awakening is critically important. These findings suggesting that early morning cortisol levels can be a reliable biological marker for baseline adrenocortical activity (Pruessner et al. 1997).

The relationship between cortisol and weight has been inconsistent, with one study showing no relationship with weight in subjects of varying ages (Pruessner et al. 1997) and another study showing a positive correlation between morning cortisol

level and weight (Kiess et al. 1995). However, the study finding the positive correlation did not control for age in the analysis, and also did not standardize morning saliva collection based on time of wake-up, using absolute time instead.

Cortisol levels show a significant increase with age from the first to the 18th year of life (Jonetz Mentzel and Wiedemann, 1993; Kiess et al. 1995). The development of the cortisol response system appears to stabilize at 6 months, as evidenced by consistent individual differences in cortisol by that age (Lewis and Ramsay, 1995). Females have been shown have higher cortisol levels than males, although not always significantly across different ages (Kiess et al. 1995; Pruessner et al. 1997).

Cortisol is responsible for many of the features of the stress response system including modulation of energy release by stimulation of hepatic gluconeogenesis, immune activity by down-regulation of the cytokine cascade, mental processes by influence on hippocampal cells, growth by inhibition of growth hormone, and reproductive functions by inhibition of gonadal steroids (Munck et al. 1984). Furthermore, complex interactions exist between the HPA axis and other neuroendocrine activities, including catecholamine systems (Axelrod and Reisine, 1984). The release of cortisol stimulates gluconeogenesis, suppresses inflammation and immune response, and affects areas in the brain relating to emotions and learning (Gunnar, 1992).

Cortisol and stress response
It has been well documented that situations associated with physical or mental exertion, novelty or uncertainty, social conflict, negative emotions and feelings of threat or loss of control result in CRH release and consequent elevations

in cortisol level (Levine et al. 1989). It is widely accepted that cortisol is increased in situations of high-ego involvement and low controllability and predictability, such as during a dental examination, anticipation of parachute jumping, final exams, or public speaking (Kirschbaum and Hellhammer, 1994). Cortisol has also been shown to rise in response to physical stress such as painful medical procedures, and physical exercise above 70% of maximal oxygen uptake (Kirschbaum and Hellhammer, 1989), and can be measured in plasma, urine, and saliva.

Salivary cortisol as a measure of stress

Salivary cortisol is an excellent indicator of stress level because it is non-invasive, is a good indicator in short-term stress situations, and is a well-established measure in people of all ages (Walker et al. 1978; Umeda et al. 1981; Hiramatsu, 1981; Vining et al. 1983; Luthold et al. 1985; Burtis and Ashwood, 1986; Al-Hakiem and Abbas, 1987; Francis et al. 1987; Laudat et al. 1988; Griffiths et al. 1989; Walker et al. 1989; Hubert and de Jong Meyer, 1989; Schreinicke et al. 1990; McBurnett et al. 1991; Gunnar et al. 1992; Gunnar, 1992; Gunnar and Nelson, 1994; Lewis and Ramsay, 1995; Kiess et al. 1995). In addition, salivary cortisol levels accurately reflect the unbound free hormone fraction, which is the fraction reaching target tissue and eliciting effects (Mendel, 1989). Although the actual concentration of cortisol in saliva is lower than in blood, salivary cortisol more closely represents the free cortisol fraction in serum than does total serum cortisol (Kirschbaum and Hellhammer, 1989), and has been shown to be a better measure of HPA function than plasma measurements (Vining et al. 1983). Salivary cortisol levels are unaffected by saliva flow rate because cortisol enters the saliva passively, by means other than an active transport mechanism (Walker et al. 1989; Kirschbaum and Hellhammer, 1994).

Cortisol levels and human behaviour

High levels of cortisol have been significantly associated with deviant behaviour, such as juvenile delinquency, army discharge, alcohol/drug use, violence, and vagrancy (Mazur, 1995), and depression (Halbreich et al. 1985; Goodyer et al. 1991; Buydens Branchey and Branchey, 1992; Roy, 1992). Among depressed adults, significant associations have been found between afternoon baseline cortisol level and having made a violent suicide attempt (Roy, 1992). Similarly, in a group composed of alcoholics with a history of either depression or violence, significant cortisol increases were found in the alcoholics who had been incarcerated for violent acts, when compared with alcoholics who had no problem with regulation of mood or aggression (Buydens Branchey and Branchey, 1992).

Correlations have been shown between cortisol levels and self-rating of mood, although the research in this area is limited. In one study, men were asked to rate their mood after watching a frightening movie. Men who described themselves as "anxious" had high levels of salivary cortisol, and those who rated themselves as "joyful" had lower levels of salivary cortisol (Hubert and de Jong Meyer, 1989).

Cortisol levels in children

A limited amount of data comes from research with children. In children aged 7-14 with disorders such as ADHD, higher cortisol levels were associated with increased levels of internalizing behaviour, and decreased reactivity (Scerbo and Kolko, 1994). Pre-pubescent children with anxiety disorder showed higher levels of salivary cortisol, with a significant interaction between conduct disorder and anxiety disorder. Children with both anxiety disorder and conduct disorder had higher levels of cortisol than children with just conduct disorder (McBurnett et al. 1991).

In second-grade children, cortisol excretion has been shown to be higher on test days than on non-test days, with the highest levels of cortisol found in children slightly above average and in those slightly below average. The cortisol levels were not related to children's self-reports of anxiety, though they were positively associated with attention to schoolwork and with positive social involvement (Tennes and Kreye, 1985). In contrast, a similar more recent study found that children who had poor school performance (measured by achievement and motivation) had lower cortisol values (Spangler, 1995). Lower values were also reported in children with type A behaviour.

A study with preschool children suggests that the relationships among cortisol, reactivity and behaviour are very complicated, and reflect a host of individual and environmental factors (Gunnar et al. 1997). Results from studies in Ethiopia (Dobrowolska and Panter-Brick, 1998), Nepal (Panter-Brick et al. 1996b), St. Vincent (Durbrow et al. 1998), and Dominica (Flinn and England, 1997) also highlight the complicated inter-relationships between socio-cultural, individual and environmental factors that contribute to cortisol levels.

Cortisol levels and animal behaviour

Research with animals indicates that increased baseline cortisol levels have been significantly associated with increased restlessness and threat response (Kojima et al. 1995). Chronic foraging demands on adult female squirrel monkeys have been shown to result in long-lasting increases in plasma cortisol levels (Champoux et al. 1993; Lyons et al. 1998).

In several studies with animals, social ranking has been strongly associated with cortisol level. Resting baseline cortisol levels were higher in sub-ordinate pigs

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when compared with dominant (Fernandez et al. 1994), rank in an established group of female rhesus monkeys was significantly negatively correlated with cortisol level (Gordon et al. 1982). Submissive male golden hamsters show elevated plasma cortisol and ACTH levels following one agnostic encounter, though the cortisol response was attenuated following repeated exposure to a dominant opponent (Huhman et al. 1991). Cortisol levels have not been shown to rise in response to isolation (Carbonaro et al. 1992a) or food thwarting (Carbonaro et al. 1992b) in dairy goats.

Catecholamine response

Catecholamines: structure and function

        Catecholamines are compounds containing an amine attached to a benzene ring that bears two hydroxyl groups. Biosynthesis and release of catecholamines is controlled by feedback inhibition. Nerve stimulation is necessary for the release of catecholamines, with chemical mediation of the release linked to aceylcholine presence and calcium ion influx. Catecholamines are released from their particles by exocytosis, requiring glycolysis or oxidative metabolism (to produce ATP). After being released from storage particles, catecholamines act on effector sites and then are rapidly inactivated in one of three ways: reuptake by storage particles, conversion to metabolites, or excretion as free amines or conjugates (Burtis and Ashwood, 1986).

        The three most important catecholamines are epinephrine (adrenaline), norepinephrine (noradrenaline) and dopamine [(3,4-dihydroxyphenyl)ethyl amine]. Epinephrine is produced by the adrenal medulla, and influences metabolic processes such as carbohydrate metabolism, whereas norepinephrine is produced by the

Ashwood, 1986).

Epinephrine

Norepinephrine

COMT
(Liver, Kidney, RBC)

MAO
(Adrenergic nerve endings)

Metanephrine
(MN)

Normetanephrine
(NMN)

Dihydroxymandelic aldehyde
(DHM aldehyde)

MAO

oxidation

reduction

3-Methoxy-4-hydroxy-
mandelic aldehyde
(MHM aldehyde)

Dihydroxymandelic acid
(DHM acid)

Dihydroxyphenylglycol
(DHPG)

oxidation

COMT

COMT

Vanillylmandelic acid
(VMA)

Methoxyhydroxyphenylglycol
(MHPG)
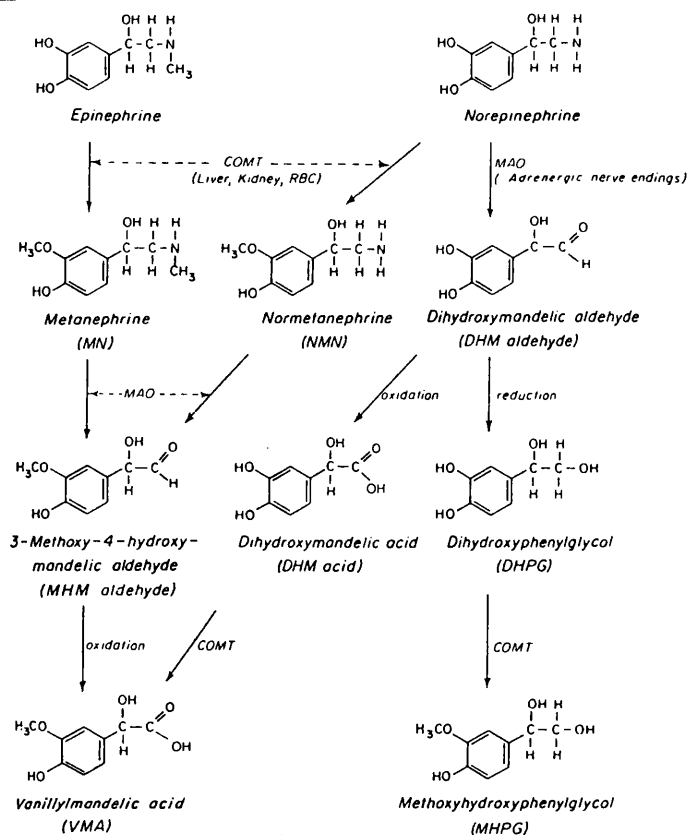
Figure 3: Metabolism of epinephrine and norepinephrine. Aldehyde intermediates exist only transiently. From Burtis, 1986: page 1743.

Catecholamine assessment

Epinephrine and norepinephrine can be assessed directly in blood

(Kozlowski et al. 1973; Dimsdale and Moss, 1980; Barnes et al. 1982; Zametkin et

al. 1985; Zametkin et al. 1987; Mansell and MacDonald, 1989; Burch et al. 1992;

Candito et al. 1993; Martineau et al. 1994) or urine (Odink et al. 1986; Zametkin et

al. 1985; Zametkin et al. 1986; Zametkin et al. 1987; Marrone et al. 1993; James et

al. 1993; Martineau et al. 1994), or indirectly via urinary metabolites (Januszewicz et

al. 1979; Abeling et al. 1984; Zametkin et al. 1985; Zametkin et al. 1985).

Epinephrine, norepinephrine and response to stressors

As discussed above, glucocorticoids and catecholamines are the most critical hormones released during the stress response (Sapolsky, 1993). Production of cortisol and epinephrine, in particular, has been linked directly with psychosocial stressors (Panter-Brick and Pollard, 1999).

In humans, epinephrine has consistently been linked with psychosocial stress, particularly time demand and work load (Panter-Brick and Pollard, 1999). In adults, heightened epinephrine levels have been associated with days at work relative to days at home (Frankenhaeuser et al. 1989; James et al. 1993; Pollard et al. 1996), and in response to short-term stressors such as public speaking (Dimsdale and Moss, 1980) or mental tests (Januszewicz et al. 1979; Barnes et al. 1982). Interestingly, epinephrine levels are higher in people working in industrialized societies when compared with those living in traditional subsistence lifestyles (Panter-Brick and Pollard, 1999).

In children, higher epinephrine levels have been recorded at day-care relative to home (Lundberg et al. 1991; Lundberg et al. 1993). Only one study shows home levels higher than school levels (Long et al. 1993), and the authors attributed the finding to the particularly structured school environment where the testing was undertaken.

Autonomic nervous system response: heart rate

Heart rate as a measurement

Heart rate was used to measure autonomic nervous system activity because it could be measured non-invasively, continuously, and accurately throughout the testing session (Manuck et al. 1990).

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Heart rate has traditionally been used to measure short-term reactivity, particularly in response to speech stressors like mental arithmetic, where responses includes increased systolic and diastolic blood pressure, heart rate, T-wave amplitude, and blood volume pulse (LeBlanc et al. 1979; Linden et al. 1985; Linden, 1987; Jorgensen et al. 1990; Frankish and Linden, 1991; Fredrickson et al. 1991; Linden, 1991; Lamensdorf and Linden, 1992; Sgoutas-Emch et al. 1994; Cacioppo et al. 1995). Children have shown heart rate responsivity to television video games (Murphy et al. 1988), going to day care (Lundberg et al. 1993), mirror image tracing and isometric exercise (Matthews et al. 1990), and postural change (Papavassiliou et al. 1996).

Although it is quite easy and convenient to measure heart rate responses to stressors, most researchers now believe that other more sensitive and specific measures are more appropriate. For example, vagal tone measurements incorporate lateral brain function with the regulation of the peripheral autonomic nervous system, which allows for a physiological analysis of the expression of emotion (Fox, 1989).

Biological correlates of inhibition

Previous work has linked altered physiological systems to particular behavioural characteristics. For example, in an American study, children were identified as extremely shy and timid when they were one year old and followed until they were seven (Kagan et al. 1988). In children representing extremes of shyness or boldness, there was a substantial positive relation between a composite of various physiological factors and the index of inhibition assigned by the research team. Specifically, the "inhibited" children were reported as having increased heart rate, levels of norepinephrine and cortisol and pupillary dilation. Although this

difference did not appear at later ages, these data have been interpreted to mean that socially inhibited children, defined as tending to restrain or restrict one's approach to new people, events, and/or objects, may have hyperactive adrenal cortical and sympathetic nervous systems, and a lower threshold for limbic-hypothalamic arousal to unexpected changes in the environment, or to novel events.

This hypothesis is strengthened by a study with 18-month olds in which elevations in salivary cortisol occurred in a group of insecurely attached and highly inhibited children in response to novelty, but not in any other group (Nachmias et al. 1996). Similarly, older shy women had higher 24 hour urinary free cortisol excretion than did non-shy women (Bell et al. 1993).

Since undernourished children have consistently been described as inhibited or anxious, they could also have abnormal adrenal cortical responses, which could diminish with their coping abilities. Strengthening this hypothesis are data about children of short stature from developed countries who have also been described as having psychosocial problems, including social withdrawal (Gordon et al. 1982; Siegel et al. 1991; Hoey, 1993).

Undernutrition and stress response

Animal model

In undernourished animals, major links have been made between poor nutrition and important functional disorders, although no research has been done with rats that are mild-moderately malnourished. Rats who had been malnourished in the pre-weaning period, and then rehabilitated had larger reactions to noise and electric shock than control rats (Levitsky and Barnes, 1970). Similarly, previously undernourished rats show stronger avoidance behaviour than controls in response to noise and electric shock (Smart et al. 1975; Lynch, 1976), and increased

emotionality or anxiety (Levitsky and Barnes, 1970). The adrenal glands of the undernourished rats were consistently larger than those of control rats (Levitsky and Barnes, 1970).

More recent work has shown that foals who received a supplemented pre-weaning diet showed better coping during weaning than did foals whose diets were non-supplemented (Hoffman et al. 1995). Plasma cortisol levels were elevated in adult female squirrel monkeys that had to look for food that was not always available (Champoux et al. 1993). Heightened plasma cortisol levels have been found in animals receiving reduced diets (Dwyer and Stickland, 1992), particularly low protein and low energy diets (Becker, 1983; Rao et al. 1968; Ausman et al. 1989).

Little is known about the mechanisms controlling physiological responses to stressors in undernourished animals, though some evidence suggests that early malnutrition induces enduring changes in central noradrenergic activity, subsequently altering motivation, emotional activity and cognitive flexibility (Levitsky and Strupp, 1995).

Severe malnutrition and physiological stress in children

Heightened free cortisol levels have also been measured in children with marasmus and kwashiorkor, although the levels return to normal after the acute stage (**Table 3**). Although most of the studies show high levels of cortisol associated with severe malnutrition, most of the studies do not control for level of infection, which may also contribute to heightened adreno-cortical activity.

Table 3: Studies about severe malnutrition and physiological stress in children

Author, yr, country	Sample, study design	Results	Conclusions
(Castellanos and Arroyave, 1961) Guatemala	n=6 K n=5 M	Higher excretion of steroids in M>K*; After 8 wks treatment K>M, NS	Higher levels during acute phase. No difference after treatment
(Alleyne and Young, 1967) Jamaica	n=36 MM No control group	High levels of plasma cortisol in malnourished Fell after recovery. (approx. 60 days)	Reduced cortisol catabolism
(Najjar and Bitar, 1967) Lebanon	n=16 M	No difference in urinary 17-OHCS (hydroxycorticosteroids) excretion	No difference
(Rao et al. 1968) India	n=22 K n=8 M n=12 C	Mean plasma cortisol levels raised in K** & M***, M>K*** Values return to normal in all children	Heightened adrenal activity associated malnutrition.
(Schonland et al. 1972) South Africa	n=15 M n=10 C	Mean plasma cortisol level higher in malnourished children*** Decreased cortisol binding capacity***	High cortisol levels when acute No differences after treatment;
(Paisey et al. 1973) Mexico	n=13 M (40% deficit in W/A) n=7 K (30% deficit W/A) n=24 C	No difference between malnourished and normal either before treatment or after 15 or 30 days.	No difference
(Prinsloo et al. 1974) South Africa	n=29 K, hospitalized No ages reported	Cortisol decreased over time, but not significant	No difference in K
(Samuel et al. 1976) India	n=9 MK n=26 underweight n=30 C	Mean plasma cortisol levels in MK< controls, NS Free cortisol levels, MK>controls*	High cortisol with acute infection Normal levels with chronic
(Olusi et al. 1977) Nigeria	n=30 K n=30 M n=30 C	Serum cortisol levels greater in both types of malnutrition than in controls**	Higher cortisol levels in M and K

(Johnson et al. 1980) Nigeria	n=29 K n=15 M n=15 controls, in hospital for minor surgery, also 1-5 y.o. but not matched	K and M > C	Higher cortisol levels in M and K
(Smith et al. 1981) Nigeria	n=16 M, K, and MM n=4 controls	K and M > MM	Higher cortisol levels in M and K
(Aref et al. 1982) Egypt	n=20 K n=13 MK n=12 M (Wellcome) n=15 controls	Hyperfunction of adrenal glands ***	Hyperfunction of adrenal glands
(Guler et al. 1992) Turkey	n=21 M n=9 K n=34 C	Increased levels of growth hormone and cortisol in both types of malnutrition when compared with controls	Higher cortisol level and growth hormone in K and M
(Zin Thet Khine et al. 1992) Burma	all male, 12-59 months old n=5 MM n=6 C	Higher levels of serum cortisol during initial period of diarrhoea, even when corrected for level of dehydration. Difference disappears with treatment	Higher levels of cortisol in MM

* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$; W/H weight for height, M marasmus, K kwashiorkor, MK marasmic kwashiorkor, C control, MM mild-moderate malnutrition:

Anorexia nervosa

Increased plasma levels of glucocorticoids have been consistently observed in patients with anorexia nervosa (Boyar et al. 1977; Doerr et al. 1980; Donohoe, 1984; Girardin et al. 1991), who generally have slower metabolic clearance of cortisol than non-anorexic controls (Boyar et al. 1977).

Conclusions

Previous work has linked altered physiological systems to particular behavioural characteristics. Specifically, children identified as extremely shy and timid had increased heart rate and cortisol levels implying that socially inhibited children may have hyperactive adreno-cortical and autonomic nervous systems, or a lower threshold for limbic-hypothalamic arousal. Undernourished children have consistently been described as inhibited or anxious, suggesting that they too might have altered hypo-pituitary-adrenal or autonomic nervous system function.

Chapter 5: Aims, objectives and hypotheses of Jamaican studies (Study 1 and 2)

Rationale

Extensive evidence indicates that children who are stunted show deficits in cognitive function, school performance and behaviour. Although direct nutritional deficiencies (e.g. energy, iron, zinc, fatty acids) certainly contribute directly to these negative outcomes, many other factors may also play a part. The purpose of the work reported here is to explore the role of impaired stress response as a potential mediating variable contributing to the poor developmental outcomes of malnourished children. It is crucial to know if children who have suffered a history of undernutrition are less able to adapt to novel environments, or become frustrated and anxious more easily than adequately nourished children. A heightened susceptibility to stress may not only contribute to the poor cognitive functioning of undernourished children, but may also affect their ability to relate to people and to cope with common external life stressors.

Goals and Aims

Thus, the aim of the reported research was to compare physiological measures of the cardiovascular, adreno-cortical, and autonomic nervous systems in stunted and non-stunted children in their baseline levels and responses to psychological stressors, based on real-life situations that occur frequently in school and home settings, and physiological stressors.

Objectives

To compare the baseline levels and reactivity of stunted and non-stunted children, the dependent variables consisted of the following physiological and behavioural response measures:

Physiological (measured over entire testing period)

    a) Heart rate: cardiovascular reactivity, and autonomic nervous system activity continuously throughout the test session.

    b) Salivary cortisol: change in functioning of the adreno-cortical system at three times during the session.

    c) Urinary catecholamines: autonomic nervous system activity

Behavioural (measured during interview and frustrating tasks)

    a) Observational ratings (inhibition, happy affect, movement, frustration, attention)

    b) Vocalizations (recorded by type and quantity)

    c) Strategies used in coping with frustrating task.

The following psychological and physiological stressors were used during the testing session:

Psychological stressors:

    a) Personal interview

    b) Oral mental arithmetic

    c) Other cognitive tests measuring information processing

    d) Frustrating tasks

Physiological stressors:

    e) Isometric handgrip (3 minutes at 30% maximal grip)

    f) Postural change

Hypotheses

The hypothesis tested was that children who had experienced early childhood growth retardation would have different physiological and behavioural stress responses than adequately-nourished children. Specifically:

1) Undernourished children's physiological and behavioural responses to psychological and physiological stressors should be different than those of well-nourished children. Specifically, stunted children should have heightened baseline levels of:

  a) salivary cortisol

  b) heart rate

  c) urinary catecholamines, particularly epinephrine

For heart rate and salivary cortisol, responsivity in stunted children should be lower than responsivity in non-stunted children.

2) Behaviourally, stunted children are more likely to:

  a) be more inhibited and frustrated

  b) be less happy, attentive or vocal

  c) use fewer coping strategies when dealing with frustration

Chapter 6: Methodology

Introduction

Two studies were performed in Kingston, Jamaica, Study 1 and Study 2, both

of which had the same procedure, and slightly different sample selection criteria.

Children in Study 1 were part of an on-going longitudinal study of stunted children

who had been followed from early childhood, whereas children in Study 2 were

identified and recruited specifically for this study. All of the methodology besides

sample selection is the same for both studies.

The methodology for Study 3, set in Kathmandu, Nepal was very different

from that used in Jamaica. Thus, the methodology for Study 3 is described in

Chapter 10, which focuses on the study conducted in Nepal.

Study site: Jamaica

Location, climate and topography

Studies 1 and 2 were conducted in Jamaica **(Figure 4)**, an island located in

the Caribbean at 18 degrees North latitude and 77 degrees West longitude, 600 miles

(965.4 km) south of Florida, 100 miles (160.9 km) southwest of Haiti and 90 miles

(144.81 km) south of Cuba. With an approximate area of 4,411 square miles

(11,000 square km), Jamaica is 146 miles long and 51 miles wide, and is the third

largest of the Caribbean islands.

Jamaica's interior is dominated by magnificent mountains, boasting a variety

of minerals, including limestone, bauxite (aluminum ore) and deposits of gypsum

and high grade calcium carbonate. In the eastern part of the country are steep crested

Figure 4: Map of Jamaica (Magellan maps)

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ridges and deep valleys, and in the center and western regions are limestone capped highlands. A narrow coastal plain surrounds the highlands and mountains.

Temperatures vary from 27°C (80°F) to 32°C (90°F) on the coasts, to 4°C (40°F) on the highest mountains, with an average temperature in most areas of 24°C (75°F). The wettest months are normally May and October and in some areas, annual rainfall is over 2,032 mm (80 inches). The mean annual rainfall island-wide is 1940mm, with Kingston experiencing 760mm per year.

Jamaica is divided into three counties, Cornwall, Middlesex and Surrey, which are subdivided into 14 parishes: Kingston, St. Andrew, St. Thomas, Portland, St. Mary, St. Ann, Trelawny, St. James, Hanover, Westmoreland, St. Elizabeth, Manchester, Clarendon and St. Catherine. Kingston, the capital city, is situated in the south-eastern coastal plain. Montego Bay, the other main urban center, is located on the northwest coastal plain.

History and racial composition

Originally populated by Taino tribes, the country was claimed for Spain by the explorer Christopher Columbus in 1494. In 1655, the British captured Jamaica from the Spaniards until 1962, when the country became an independent member of the British Commonwealth. The majority (96%) of Jamaica's 2.5 million people (Jamaica embassy web page, 1995) are African descendents of the slaves brought to work on sugar plantations in the 17th and 18th centuries, although there are substantial populations of people of European (1%), East Indian (2%) and Chinese (1%) origin (**Figure 5**). Although English is the official language, most people speak an English-based Creole-patois.

Figure 5: Jamaican school-children in school yard.

Economic indicators

Gross National Product (GNP) per capita has been estimated at $1600

(UNICEF, State of the World's Children, 1999). Total Gross Domestic Product

(GDP) was US$4.1 billion, up from only US$3.0 billion in 1976

(www.worldbank.org). Average annual growth of GDP was estimated at -2.4 for

1997, but is projected to be 0.8 during 1998-2002. The percent of the population

below the national poverty line was estimated in 1997 as 34%. Jamaica's economy

is focused primarily on the tourist trade, with 56.9% of the GDP coming from

Services. Jamaica's imports of goods and services was 66.4% of the GDP in 1997,

almost double what it was in 1976 (www.worldbank.org).

Public health issues

The total population as estimated for 1997 by UNICEF in the State of the

World's Children (1999) was 2.515 million, and the growth rate from 1980-1996 has

been estimated at 1.1% by the World Bank (www.worldbank.org). For 1997, UNICEF estimates the under-five mortality rate at 11 per thousand, the infant mortality rate at 10 per thousand, and the annual number of under-five deaths to be 1000 (State of the World's Children, 1999). Life expectancy at birth is estimated at 75 years, adult literacy rate is 85%, 86% of the population has access to safe water, and 100% of the urban population has access to adequate sanitation (State of the World's Children, 1999).

Criteria for sample selection

Study 1, longitudinal

Background

Children were initially identified in 1984-5 when they were 9-24 months old in a neighborhood survey of poor areas of Kingston, Jamaica. All areas of Kingston were surveyed, except for those where there was a particularly high crime rate that may have put the investigators at risk. In addition, areas were excluded if they had a large percentage of commercial buildings, if other community projects were being carried out in area, or if the communities were so closely knit that assignment to different groups would have difficult. The surveyed areas were: Hope Tavern Kintyre, Hope Flats, Cockburn Gardens, Balmagie, Whitehall, Waterhouse, Allman Town, Granklyn Town, Rae Town, Central Kingston, Greenwich Town, and Spanish Town Road (**Appendix I**).

All children aged 9 to 24 months old in the surveyed areas had their lengths measured. Children who were less than minus two standard deviations from the mean height for age (n=132, <-2.0 S.D. height/age) according to National Health Center Standards (NCHS) (Hamill et al. 1977) were identified and weighed. All

these weights and heights, less the median, were then stratified by sex and age (under 16 months, and 16 months or over). They were then randomly allocated to one of four treatment groups: stimulation, supplementation, both (supplementation and stimulation) or control (no treatment).

A group of non-stunted, comparison children (n=32, >-1.0 S.D. height/age), matched to the stunted children were selected. The child was chosen who lived closest to each experimental child, and was the same sex and age (+/- 3 months). Exclusion criteria included having a birthweight less than 1.8 kilograms, any signs of severe malnutrition (Gomez classification) or oedema, hospitalization, multiple births, mental or physical handicaps, housing above certain standards (no inside toilet or water supply, or crowding of more than 2 persons per room), maternal education levels above having taken secondary level exams, in full time day care, or with no adult consistently at home prepared to work with the intervention visitors.

The stunted children, divided into four treatment groups, were compared with non-stunted children in terms of their psychomotor development starting at enrollment, continuing for the two-year intervention (Grantham McGregor et al. 1991). At first, the stunted children had lower developmental quotients than did the non-stunted group, and the non-intervention group declined during the study. However, by the end of two years, both supplementation and stimulation had independently benefited the developmental quotients of stunted children and the combination of supplementation and stimulation had an additive effect. Supplementation benefited the children's growth whereas stimulation did not (Walker et al. 1991). The children were assessed again when they were 7-8 years old, and the children in the intervention group had higher scores than control subjects on more tests than would be expected by chance, suggesting a very small

global benefit of the intervention (Grantham McGregor et al. 1997). The additive effect of stimulation and supplementation had disappeared.

<u>Sample composition</u>

The sample of Study 1 was comprised of two groups, stunted and non-stunted.

Subjects (stunted): All children from the original group whose heights remained below -1.5 S.D. at the time of testing, and who remained in the Kingston area (**Appendix I**) (n=30).

Controls (non-stunted): Children who were originally non-stunted children, whose heights remained above -1.0 S.D, who also remained in Kingston (n=24).

Study 2, cross-sectional

Subjects and controls were identified from schools in poor areas of Kingston (**Appendix I**). All children aged 102-120 months in grades 2, 3, and 4 were measured from the government schools in downtown Kingston (**Table 4**).

Table 4: *Schools in downtown Kingston used for screening and enrolling eligible children for study 2*

School name	Location	Total in study
New Providence	361 Barbican Road, Kingston 6	2
Seaward All Age	119A Olympic Way, Kingston 11	0
Denham Town Primary	105 North Street, Kingston 14	8
Drews Avenue Primary and Infant	Drews Avenue, Kingston 20	8
Clan Carthy Primary	5 Deanery Road, Kingston 16	2
Balmagie Primary	131 West Bay Farm Road, Kingston 11	6
Holy Family Primary and Infant	2 Laws Street, Kingston	10
Franklyn Town Primary	4 Victoria Street, Kingston 16	8
Rollington Town Primary	16 a-b St. James Road, Kingston 2	0
St. Aloysius Primary	76 Duke Street, Kingston	0
St. Peter Claver Primary	33B Waltham Park Road Kingston 13	6
St. Anne's Primary	71a Bond Street, Kingston C.S.O.	4
Maxfield Park Primary	51 Langard Avenue	2
Whitfield All Age	20 Whitfield Avenue, Kingston 13	2
North Street Congregational Primary	28 North Street, Kingston	4

In all schools, children were organised in "streams" according to academic achievement, with the higher achievers in the top streams. All children in each stream of each grade were screened and measured using a portable stadiometer and standard measurement techniques, and age was obtained from the teachers' records. Approximated z-scores were calculated using a table of NCHS cut-off values.

<u>Subjects(stunted)</u>: Children with height for age z-scores (Hamill et al. 1977) of less than -2 S.D. away from the mean were identified as subjects (n=31).

<u>Controls (non-stunted)</u>: Child of the same sex, from the same class with the closest birthday (within +/- 4 months), and a height above -1.0 S.D. of the mean z-score height (n=31).

If two children had the same birthday, then the child closest alphabetically to the index child was selected. If there was no child in the same class as the index child who was the same sex and age as the index child, then a child from the same stream of the next grade below was selected. This selection technique was utilized to avoid biasing the control group to include higher achieving children. After the children were identified, permission forms were sent home with the children, and after written permission was granted from the parents and verbal consent was received from the teachers, the children were enrolled for testing.

Ethical considerations

A Single Project Assurance (#S-5558-05) was granted by the Department of Health and Human Services of the National Institutes of Health Office for the Protection from Research Risks, and ethical approval was also granted by the Institute of Child Health and Great Ormond Street Hospital for Children NHS Trust Ethics Committee (R & D Reference Number 96CH13). Human subjects approval was obtained from the University of the West Indies Ethical Committee, and informed written consent was received from the parents of all children participating in the study. Great care was taken to ensure that no child would be distressed by the testing procedure. All children who participated in the study were given food, a drink, and a present of school supplies (notebooks and pencils).

General methods for Study 1 and 2

Testing session

All children were assessed in a small room at the Tropical Metabolism Research Unit, at the University of the West Indies, in Kingston. Testing occurred between January and July 1995, beginning between 8:30 and 13:30, with no significant differences between groups in terms of timing. The test session (approximately 1.5 hours) included three baseline periods when heart rate was measured, postural change, an interview, three cognitive function tests, two frustrating tasks, and an isometric hand grip test (**Figure 6**). Immediately before the first measurement of baseline heart rate (lying), all children were given 250 ml of a chocolate drink containing 229 calories, 13g of protein, 6g of fat, and 34g of carbohydrate to ensure that they were not hungry during testing, and to equalize potential post-prandial effects. Two baseline heart rate periods (one lying and one sitting) preceded the psychological testing, with the heart rate of the child moving from lying to sitting measured between the two baselines.
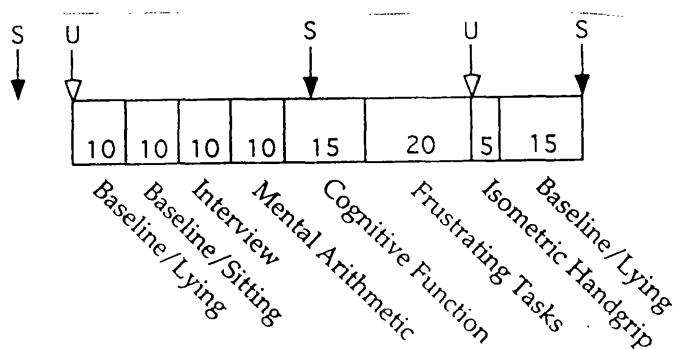
Figure 6: Chronological details of testing session, S=saliva sample, U=urine sample

Rationale for choice of stressors

Introduction

Criteria were developed in order to assess the potential use of various psychosocial tasks to be used as stressors. Stressors that have been used by other researchers were evaluated, and a list of requirements was established. In order to be considered, a task had to:

- require verbal or behavioural response from the child;

- provide one of a range of stressors, including cognitive, interpersonal, fine motor skills, quick mental processing;

- be engaging for an 8-10 year old child;

- be realistic and ecologically valid so that the results might be more interpretable;

- range in difficulty, and allow for each child to perform at her/his maximal stress level, although the levels would vary from child to child;

- provoke responsiveness in heart rate, cortisol and epinephrine;

- be culturally appropriate for a population of Jamaican school children.

Piloting

Extensive piloting occurred from October 1994-January 1995 with children of the appropriate age who attended a school that was near to the University and rot part of the selection pool for the actual study. The population of children attending the school was similar to the sample population in many aspects, particularly socio-economic status. Multiple tests were piloted in order to assess their potential usefulness. When tests that had been traditionally used were deemed inappropriate, new tests were designed to fit the selection criteria.

Extensive observations of behaviours were made in order to create ratings that were an accurate reflection of the population distribution. Based on previous research with undernourished children, particular behaviours were identified (e.g. inhibition, vocalization, movement, frustration), and were quantified based on observation. Other behaviours were added after observing children in the pilot situation (e.g. happy affect). In all cases, the ratings were repeatedly refined, so that their descriptions and distributions would be as clear and reliable as possible.

Some tests (e.g. mental arithmetic) were calibrated so that they would reflect as much of the population distribution as possible. During piloting, the testers practiced collecting saliva and urine samples, and using the heart rate monitor. Towards the end of the piloting period, children were selected to be tested at the University in order to pilot the testing period in its entirety.

Psychological stressors

The psychological testing session comprised an interview, mental arithmetic, a modified Symbol modalities test and a pegboard test, all of which are described in detail below; the testing protocol is shown in **Appendix II**. The recording sheet for use during testing is shown in **Appendix III**.

Interview

The psychological testing began with a personal interview, designed to be a modified type of public speaking, known to increase cortisol levels (Kirschbaum and Hellhammer, 1994). An unfamiliar female adult tester (one of two testers who tested all children in both studies) sat directly across from the child. She asked a set of twelve questions, six "non-troubling" questions about school and social roles, and six "troubling" questions about problem situations. The questions were asked in a

direct manner, and the testing situation was designed to be mildly discomforting for the child.

The questions asked were designed to heighten physiological levels of stress (e.g. salivary cortisol, urinary epinephrine, and heart rate). They were also designed to provoke answers that would be revealing in terms of ascertaining the amount of violence and aggression children experience at school and home, given the importance of violence as a public health concern in Jamaica. The interview was clearly designed primarily with the first goal in mind, of using the questions as a stressor. Thus, deep and/or meaningful interpretation of the interview responses is limited by the fact that the study was not designed to generate either data about interview content that can be generalized across other populations.

The questions six non-troubling questions about social roles (1-6 below) and six troubling questions about problem situations (7-12 below):

1. "What happens at school?"

2. "What does a teacher do?"

3. "What does a principal do?"

4. "What does a father do?"

5. "What does a mother do?"

6. "What does a policeman do?"

7. "What happens if you lose your lunch money?"

8. "What happens if you lose your school book that your mother gave you?"

9. "What happens if you tear your uniform?"

10."What happens if you're late for school?"

11."What happens if you get into trouble at home?"

12."What happens if someone that you know from your class takes your

money?"

After asking the question, the interviewer waited for 10 seconds for the child

to begin responding; if the child did not respond, the interviewer prompted the child

again. If the child did not respond within 10 seconds to the second prompt, then the

interviewer rated the child's answer as "No Response" and continued with the next

question.

If the child began talking at any time after the question had been posed, the

interviewer recorded the response. When the child stopped talking, the interviewer

waited for 10 seconds, and then asked, "Anything else?" If there was a response at

that time, it was recorded in a separate space on the testing sheet. If there was no

response, or the child shook her/his head to indicate, "No," that was also noted. The

child was allowed two minutes to answer each question, with five seconds of silence

between each question. While the child responded, the interviewer recorded the

answers onto the testing sheet.

Mental arithmetic

A mental arithmetic session was then used because it typically evokes an

immediate physiological response (Lamensdorf and Linden, 1992). Mental

arithmetic is easy to implement without instrumentation, raises few ethical concerns,

and is a good short-term stressor because it invokes an immediate response. Many

studies have shown stress response to mental arithmetic, and the response includes

increased systolic and diastolic blood pressure, heart rate, T-wave amplitude, and

blood volume pulse (LeBlanc et al. 1979; Linden et al. 1985; Linden, 1987;

Jorgensen et al. 1990; Frankish and Linden, 1991; Fredrickson et al. 1991; Linden,

1991; Lamensdorf and Linden, 1992; Cacioppo et al. 1995). The amount of increase

depends on other variables such as how the test is delivered or whether the person is hypertensive. Mental arithmetic has also been shown to increase plasma catecholamines (LeBlanc et al. 1979; Januszewicz et al. 1979; Jorgensen et al. 1990; Cacioppo et al. 1995).

At the start of the mental arithmetic, the child's competence level was established by giving a diagnostic test, so that the 30 test questions were at the appropriate level for each child (with each child answering approximately 2/3 correctly). This ensured that each child had a mental arithmetic test appropriate for her/his level, and that the test inspired a similar stress level in children, regardless of their intelligence level, or their experience with mental arithmetic in school.

All addition problems were vocally administered. The interviewer began the mental arithmetic exercise by making sure that the subject understood exactly what "adding" or "sums" was ("We are going to do some maths. Do you do adding at school?"). She confirmed the understanding by asking if the child could do 1+1, and then 1+2. If after these examples, the child seemed to understand, as indicated by answering two in a row correctly, then the interviewer proceeded with the first diagnostic test.

Three diagnostic tests (A*, B*, C*) and three actual tests (A, B, C) were designed (**Appendix III**). All children were tested first with diagnostic test A*. If the child answered 0-3 correctly out of 5, then s/he was tested with 30 questions at that level. If the child answered 4-5 correctly of A*, then the tester administered diagnostic test B*. If the child answered 0 of B* correctly, s/he returned to level A, and was tested with those 30 questions. If the child answered 1-3 correctly of B*, s/he was tested with 30 questions from level B, and if s/he answered 4-5 of B*

correctly, then diagnostic test C* was administered. For the children who answered 0 correctly on diagnostic test C*, test B was used. For those who answered 1 or more correctly at level C*, the testing occurred using the 30 questions at level C.

For both diagnostic and actual mental arithmetic tests, the tester explained that the child should answer "quick, quick" and do the sums without counting on fingers. For all tests, the child was allowed 5 seconds to answer each question. If the answer was correct, the tester said nothing; if the answer was wrong, the tester said, "That's wrong" and if the child was too slow in answering, the tester said "Too slow." All answers were recorded on the testing sheet.

Symbols

Thereafter was a modified Symbol Digit Modalities test (Symbols) (Smith, 1973), measuring information processing speed and paired associate learning which was repeated twice (**Figure 7**). The test consists of a page of squares and hexagons organised in 8 lines in a random order (**Appendix IV**). At the top of the page, a square is pictured with a book inside it, and a hexagon is pictured with a pencil inside it; the rest of the squares and hexagons on the page are empty. The idea was for the child to point at an empty square and say "book" and to point at an empty hexagon and say "pencil." Since the shapes are not in systematic order, the test requires the child to register the shape quickly, translate the shape into an object, say the name of the object, and then move along at a rapid pace. The tester used three different sheets, each with a different arrangement of squares and hexagons. The first sheet was used for explaining and practicing, and the other two were used for testing. The three sheets were always used in the same order.
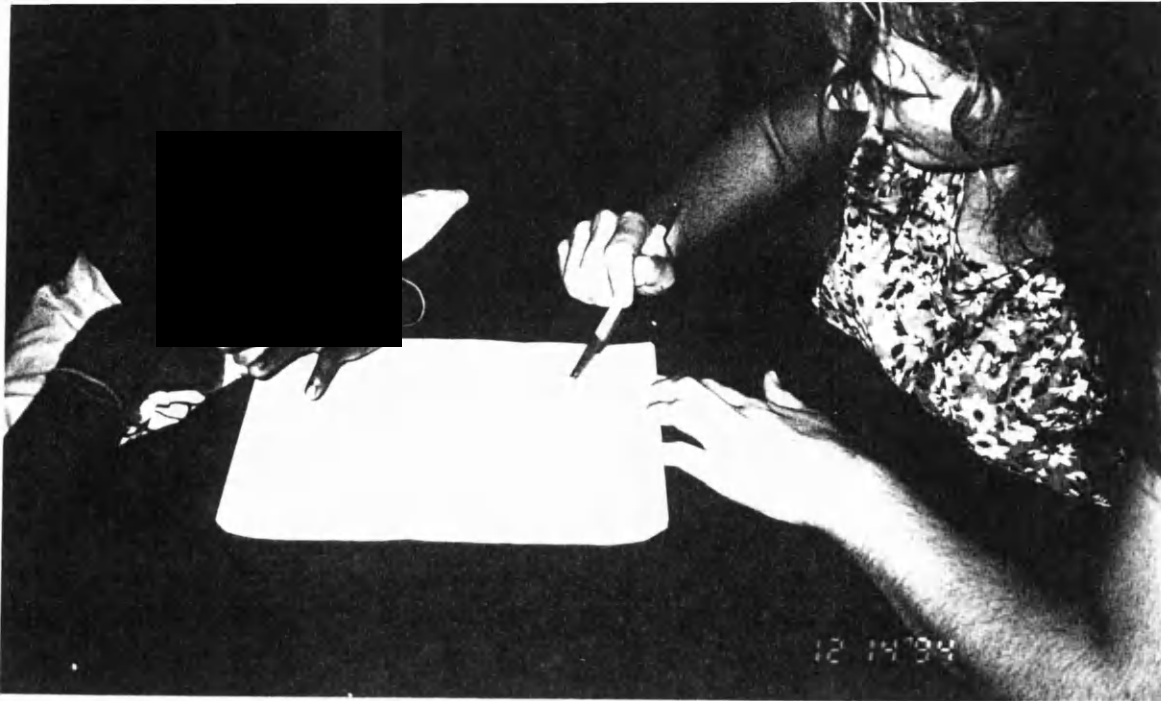
Figure 7: *Tester administering Symbols test*

The child practiced one line with the tester, and then tried the test alone. The tester waited to continue until it was clear that the child understood, and that s/he completed three lines with 2 or less mistakes. The tester then instructed the child to do the whole of the first test page. For each test page, the tester recorded beginning and ending times, and the number of errors per page. At the beginning of every third line (three times for each page), the instructor said "Quick, quick" to the child.

Pegboard

The pegboard test (Lafayette Instrument Company: Lafayette IN), measured fine motor function and was repeated twice. The test setting during the mental arithmetic, symbols, and pegboard tests was designed to be ecologically valid; the children were under time pressure, mimicking the standard school examination setting. The pegboard consisted of a board with a grid on it, into which little metal "keys" fit. The "keys" were all exactly the same, but the holes into which they were supposed to be placed were arranged in alternating orientation. Thus, it was

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necessary for the child to observe the orientation of each hole, and then fit the key into each hole accordingly.

The tester let the child practice on a minimum of 24 holes, and then when the practice effect seemed to have disappeared, the test was started. All children were given a treat regardless of their performance on the test.

Frustrating tasks

In the final part of the psychological testing, subjects were presented with two frustrating tasks, which were designed particularly for this study. Several frustrating tasks were piloted, and the chosen tasks were frustrating enough to provoke responsiveness, but interesting enough so that the child would not give up right away. Both tasks were virtually impossible, but seemed to have simple and attainable solutions.

Beads

The first task involved threading clogged beads onto a 12-inch piece of embroidery thread. Of the 20 beads that were offered, only one was actually threadable, as the remaining beads were clogged with invisible glue. To start with, the tester gave the child a piece of thread, and an unclogged bead, so that they could have the satisfaction of threading one bead successfully. The children each had 10 minutes to work, and were each given a beaded thread at the completion of the exercise (**Figure 8**).

Figure 8: *Child receiving the gift of a beaded thread at the end of the bead task.*

Etch-a-Sketch

The second task involved drawing a circle on an awkward apparatus (Etch-a-Sketch, Ohio Art Company: Bryan OH). The child was presented with an Etch-a-Sketch, which is a toy used for drawing. Two knobs, each of which could move the line either horizontally or vertically, governed the movement of the "pen." To get a curved line, it was necessary to move the knobs simultaneously, and it was extremely difficult to draw a smooth, curved line. The tester explained how the toy worked, demonstrated some lines, then showed the child a picture of a square in order to practice using the knobs. After the child was sufficiently familiarized with the apparatus, the tester asked the child to try and draw a circle. The child's behaviour was then observed for the next five minutes, or shorter depending on whether the child gave up.

In both frustrating tasks, the child was not allowed to stop engaging completely in the activity, and was encouraged to keep trying until at least half of the

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test session was over. After half of each session was finished (5.0 minutes for beads, 2.5 minutes for Etch-a-Sketch), the children were allowed to stop at will.

Physical stressors

Traditionally used as alternatives to dynamic exercise for measures of cardiovascular responsiveness, postural change and the isometric handgrip were used as physical stressors in this study.

Postural change

Postural change is a commonly implemented physical stressor c.f. (Goldstein and Shapiro, 1995; Ng et al. 1995; Musante et al. 1995; Papavassiliou et al. 1996) that results in acute decreases in intravascular plasma volume, and hemoconcentration of serum lipids and plasma proteins. Postural change was measured when the children switched from the lying baseline to the sitting baseline before psychological testing. After the lying baseline was completed, the tester asked the child to sit up on the bed, and then get off the bed to stand on the floor. The child then sat directly in a chair that was placed next to the bed in order to complete the sitting baseline.

Isometric Handgrip

The isometric handgrip is commonly used as a method of measuring human response to physical stress c.f. (Lind et al. 1964; Jayarajan et al. 1985; Jones et al. 1986; Jayarajan and Shetty, 1987; Misner et al. 1990; Jayarajan and Shetty, 1992; Smith et al. 1993). To measure handgrip, a sphygnamometer was used because it was easy for the child to squeeze. After the final baseline was completed, the testers ascertained each child's voluntary maximum grip strength by asking her/him to squeeze as hard as possible for five seconds. Then, the children grasped the

handgrip for two minutes at 30% maximal grip strength (Jayarajan and Shetty, 1992).

Outcome measures

Salivary cortisol

Three saliva samples of approximately 0.5 ml each were obtained from each child (**Figure 6**). The first sample was a baseline measure and was taken on arrival at the University and before the children were given a drink. The second sample was linked to the first part of the psychological test session, and the third to the second part of the psychological test session, based on an approximate 20-25 minute time delay between cortisol secretion and subsequent salivary levels (Gunnar and Nelson, 1994). All samples were collected in sterile collection tubes (Salivettes, Sarstedt: Newton, NC) centrifuged briefly (1000g, 2 minutes, 20°C), transferred to cryovials, and then frozen at -80C. The samples, on dry ice, were shipped directly to the lab at Stanford University where they were assayed.

I evaluated the cortisol levels by using a modified (Kirschbaum et al. 1989) I-125 radio-immuno-assay kit ("Magic Cortisol," Ciba-Corning: Medfield, MA) in a laboratory where this assay was regularly performed (**Appendix V**). Intra-assay coefficient of variation (±S.D.) of duplicate samples was 3.1% ± 2.1, and inter-assay coefficient of variation of a standard saliva pool was 5.6% ± 3.9 (n=7). Cortisol values at the three time points were moderately inter-correlated (range r=0.32-0.76). To control for the known diurnal rhythm in cortisol levels in the study design, time of testing was balanced across children from both groups. Complete salivary results were available for 50 of 54 children (93%) in Study 1 and 65 of 68 children in Study

2, with 121 of 122 children (99%) having at least two of the samples, and losses due to insufficient saliva.

Heart rate

Heart rate was recorded throughout the session via a chest band (Polar Vantage XL heart rate monitor, Polar CIC Inc.: Port Washington, NY), that has been used frequently to assess heart rate (Ballarin et al. 1989; Tono oka and Kaneko, 1993; Loftin et al. 1996). Children were carefully fitted with the monitor so that it would not be too tight (**Figure 9**).

Before being used in the testing situation, the heart rate monitors were validated with simultaneous measurement by ECG at the Cardiology Unit of the University of the West Indies (**Table 5**), and were shown to be highly valid (Pearson, $r=0.994$, $p<0.0001$).

Figure 9: Child being fitted with the Polar Vantage XL Heart rate monitor

Table 5: *Validation of Polar Vantage XL heart rate monitor with ECG at the Cardiology Unit, University of the West Indies.*

Subject age (yrs), sex	ECG, Unit	Polar Vantage XL heart rate monitor
	beats /minute	
23, female	74.58	74.00
22, male	90.69	88.50
22, female	53.06	53.80
20, female	73.52	75.63
23, female	78.48	78.57
22, female	82.97	84.07
23, male	55.14	53.89
21, male	71.78	71.46

The baseline heart rate results were also shown to be valid over time. This measure of reliability was confirmed by comparing the same children two weeks apart, in the same sitting position ($n=11$, $r=0.805$).

The Polar Vantage XL heart rate monitors are designed with a recording belt and a watch, where the information is stored, which must remain within three feet of the belt. For this study, the watches were set to record average heart rates for every 5 seconds, which was the most accurate setting possible. At the beginning and end of each section of testing, the tester pressed a button on the watch, and the exact time was then be recorded inside the watch.

After each test, the data for each child were downloaded into a Macintosh portable computer, with a commercially available computer interface device (Polar CIC Inc.: Port Washington, NY). The data consisted of a list of heart rates (12 values for each minute), and a list of "intermediate times," comprising the exact time

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of starting and finishing each sub-section of the testing (approximately 40 values). In a very few cases, the child being tested would push the "intermediate time" button on the heart rate monitor watch. For this reason, approximate times for the start and finish of each test section were also recorded by the tester onto the testing sheet, so that the real times could be confirmed during data analysis.

The listing of intermediate times was then compared with the test record, in order to determine the most accurate listing. These values for the beginning and ending times of each sub-section (test events and the time in between events) of testing were then entered manually. This information was then transferred along with the heart rate data directly into a program that was created in Hypercard (PolarCalc0.2: Stanford, CA), and is not commercially available. The program was designed to calculate duration, mean, standard deviation, range, maximum and minimum values for each event.

Full baseline and psychological heart rate results were available for 53 of the 54 children (98%) in Study 1 and all children in Study 2. Results for a sub-sample of 41 of 54 children (76%) in Study 1, and 66 of 68 children (97%) in Study 2 were available for postural change.

Urinary catecholamines

Two urine samples were obtained, one 15 minutes before onset of the interview, and one at the end of the testing period. According to standard procedure (Burtis and Ashwood, 1986), samples were acidified within 1-2 hours of testing with 6 M NaCl to a pH of 2, and then stored at -80C. Only the urine samples collected at the end of the testing period were assayed.

Samples were shipped on dry ice to be assayed at the University of Kwazulu Natal, South Africa, according to a standard method (Odink et al. 1986) (**Appendix**

VI). Ion-pair reversed phase HPLC was performed, and the values were determined by electrochemical detection using a glassy carbon electrode at a potential of 600 mV (vs. Ag/AgCl reference). Results were corrected for the recovery of the internal standard. Results were also corrected for the volume of urine produced because they were reported per volume creatinine.

Measures of inter- and intra- assay variation for catecholamine (norepinephrine, epinephrine and dopamine) are shown in **Table 6**.

Table 6: Inter-assay and inter-assay variation for urinary catecholamine analysis

Coefficient of variation (%)	Norepinephrine	Epinephrine	Dopamine
Intra-assay	3.1	5.8	2.1
Inter-assay	2.4	3.8	2.2

Reference values were based on a reference range of 496 consecutive routine laboratory samples (500 samples, of which 4 were rejected as outliers). Since the catecholamines in this study were from children rather than adults, the reference values were modified for children according to standard procedure.

Behavioural observation

Throughout the interview and frustrating tasks, behaviours were observed and rated by one of two testers on 5-point scales (Barrett, 1987). The behaviours were selected based on previous observations of undernourished children (Barrett and Radke-Yarrow, 1985; Meeks-Gardner et al. 1997), and on previous research linking inhibition and stress reactivity (Kagan et al. 1988; Kagan, 1997). As described, the ratings were carefully calibrated during piloting, and each point on the rating had a specific definition (**Table 7**).

Behaviours were rated twice during the interview (once after the first question was asked, and once after the last question was asked), and included responsive vocalizations, happy affect, inhibition, and movement. Behaviours were also rated once during each frustrating task (beads and Etch-a-Sketch) and included affect and movement, which were the same as in the initial interview, and frustration, and attention. Spontaneous vocalizations were recorded according to number and type of response made (see **Table 8** for coding).

Table 7: Coding for behaviour ratings for interview and frustrating tasks

Rating	Behaviour
Vocalization	
1	No Response, or "nothing"
2	Single words, not joined together in sentences, or, 1 three or four word sentence
3	≤ 3 incomplete sentences, or, ≤ 3 short complete sentences, or, one long sentence with 3 or less verbs.
4	Two or four longer sentences, or, greater than three short sentences, or, one long sentence with more than three verbs.
5	More than or equal to 5 longer sentences, with details
Inhibition	
1	Very withdrawn (avoiding eye contact with tester, eye contact that occurs is fleeting and infrequent (0-2x), clearly very uncomfortable
2	Fairly withdrawn (fairly uncomfortable, more than fleeting eye contact, but less than half of the session)
3	Neither withdrawn or outgoing (substantial eye contact (more than half the time))
4	Fairly outgoing (eye contact most of the time, fairly comfortable
5	Very outgoing (eye contact all of the time, with few lapses, clearly comfortable)
Movement	
1	No fidgeting, absolute stillness, barely any movement
2	Minimal movement (clearly still, but not completely frozen)
3	Normal movement, no "unusual" behaviour
4	Some fidgeting (obvious movement, but less than 75% of the time)
5	Lots of fidgeting (small movements all the time, or big movements more than 75% of time) Fidgeting defined as: fiddling with clothes, face, hair, hands touching face, sucking on fingers, chewing on pencil, shuffling feet
Happy Affect	
1	Clearly unhappy, not enjoying situation, upset, anxious
2	Somewhat unhappy, occasional furrowed brow or grimace
3	Neither unhappy or happy, exhibiting characteristics of neither
4	Somewhat happy, occasional smile, grin or laugh
5	Clearly happy, smiling, enjoying situation
Frustration	
1	No signs of frustration
2	Some signs of frustration, few, scattered
3	Somewhat frustrated, (<50% of time)
4	Several signs, 50-75% of time
5	Many signs of frustration, more than 75% Frustration defined as looking up at tester, grimacing, sighing, shrugging

Table 8: Examples of spontaneous vocalizations and how they were coded during the frustrating tasks

Type of vocalization	Examples of utterances
Self doubt	"can't do it," "mi spoil it"
Task failure	"this can't go on," "it cannot draw"
Help or information requested	"show me" "how should I do it?"
Exasperation	"tcha," grumbling, sighing
Other task-related comment	"I'll try for last, last time"
Other non-task related comment	"I had porridge for breakfast"

During the beads task, the testers also recorded the strategies used (**Table 9**).

The recording sheet used to keep track of strategies is shown on the last page of

Appendix III.

Table 9: Strategies recorded during the bead task and their definitions

Strategy	Definition
Lick or suck	Licking or sucking thread to make a sharp point
Divide	Dividing the embroidery thread into 2 or more thinner threads
Twist	Twisting the thread to make a sharper point
Smooth	Smoothing the thread without twisting or licking
Change ends	Changing ends of the thread
Change thread	Trying to use the other piece of thread given
Clear bead	Blowing into the bead, or picking at it with fingernails
Look in bead	Peering inside bead to see what is stuck inside
Use bowl	Using the bowl to sort beads that have already been tried from beads that have not
#X change bead	Number of times changing beads

All ratings were established during extensive piloting, and inter-observer

reliabilities were high for all measures (**Table 10**).

Table 10: *Inter-observer reliabilities for the two testers during the interview (beginning and end) and frustrating tasks (beads and Etch-a-sketch)*

Section of test	Behaviour	Spearman rank correlation
INTERVIEW (N=10)	Happy affect 1[1]	0.935
	Inhibition 1	0.970
	Movement 1	0.840
	Happy affect 2	0.925
	Inhibition 2	1.000
	Movement 2	0.778
	Responsive vocalization	1.000
FRUSTRATING TASKS (N=21)	Attention 1	0.993
	Frustration 1	0.965
	Movement 1	0.751
	Vocalization 1	0.999
	Attention 2	0.959
	Frustration 2	0.984
	Movement 2	0.917
	Vocalization 2	0.985

[1] Interview, 1=beginning, 2=end; Frustrating tasks, 1=beads, 2=Etch-a-sketch

The two testers each tested approximately equal numbers of children in each group and were blind to the child's group, which was possible because the large age range of the children masked any obvious height differences. The psychological test sessions were recorded on audio- and videotape.

Full behavioural data were available for 53 of 54 children (98%) in Study 1 and for all children in Study 2.

Home visits

The mothers or guardians of all children were visited at home, and details of maternal characteristics, including education, occupation, standard of housing, and household possessions were recorded (**Appendix VII**). The level of stimulation in the home was also assessed. The socio-economic assessment used in Study 1 was completed in the year before the reported research was completed, and the

assessment for children in Study 2 was completed simultaneously with testing. The interviewers used in the two studies were different, but both interviewers used the same questionnaire, and were blind to the child's group, trained by the same people, and not involved with any other part of the study.

To quantify socio-economic status, two indices were created that have been frequently used in Jamaica, and are associated with child development and school achievement (Walker et al. 1994; Clarke et al. 1991; Grantham McGregor et al. 1997). The economic index included "possessions," which ranged from 0-4 and reflected the existence of a television, refrigerator, radio, stove, and "crowding," which reflected the number of people per room in the house. "Crowding" was positively skewed, so the two variables, "possessions" and "crowding" were first normalized by transformation to standardized scores and then added together to create the economic index. Due to missing data in Study 2, the "possessions" component of the index in Study 2 ranged only from 0-3 because it did not include refrigerator. For the analyses of groups combined, the indices from Study 1 were recalculated to match those used in Study 2.

The stimulation index included measurements (11 in total) of the number of exercise books, school books, coloring books, paper, pencils & crayons, and toys in the home, and games, trips and verbal stimulation (i.e. reading, telling stories, teaching) from the parents. Each variable was reduced to a three-point rating, and then the variables were added together. The stimulation indices used in studies 1 and 2 differed slightly from each other, due to missing data in Study 2 for the "paper" and "teaching" ratings. Thus, for analyses of the groups combined, the index for Study 1 was re-computed to match that used in Study 2.

Socio-economic information was available for all children in Study 1 and 61 of 63 children in Study 2, due to difficulties in locating the children's homes. In order not to lose those children (n=2) who were missing socio-economic variables from the analyses, mean values from the appropriate sub-groups for the final two ratings were used to replace the missing values.

Maternal PPVT (Peabody Picture Vocabulary Test (Dunn, 1965)) and subject PPVT and subject IQ (Stanford Binet (Thorndike et al. 1986)) were also assessed for the children in Study 1. Whereas the IQ's for children in Study 1 had been obtained as part of the longitudinal follow-up in the previous year, the PPVT's for children in Study 2 were obtained by the testers in the study at the University immediately after the testing was finished. Since the PPVT score depends on what age the test was taken, it was not possible to compare scores on the PPVT across the groups from Study 1 with the children from Study 2.

Birthweight was obtained from mothers or guardians. Seventy-two percent of the hospital birth records for children in study 1 were subsequently traced as part of an additional study of the children followed longitudinally, and the mother's history was found to be a highly valid measure of birthweight ($r = 0.85$) (Gaskin et al. 1997). Birthweight was available for all children in study 1, but for only 54 of 68 children (79%) in study 2.

Current weight and height were measured using standard procedures (Lohman et al. 1989). Age was obtained from school and birth records where available. Anthropometric data were available for all children in both studies.

Content of interview responses

During the interview, responses were recorded by hand in standard written English and Jamaican patois, whenever possible, in order to retain the closest

possible representation of words used. The interviews were then transcribed into a

standard computerized word processing program (Microsoft Word 6.0 for Windows:

Redmond). Vocalizations that were missed during the manual recording were

picked up on audio-tape and added to the transcription after the interview.

Interview data were analyzed using a commercially available software

package for qualitative data analysis (QSR NUD*IST 4 for Windows: Victoria,

Australia). According to standard procedures (Dey, 1993), a portion of the interview

answers were initially coded manually, which allowed for formation of the coding

index (**Appendix VIII**). A post-hoc methodological comparison was completed,

and showed no differences between those interviews coded on the computer and

those coded by hand. Responses were retained in their entirety so that the context of

each response could be examined. Thus, each response was coded into as many

applicable categories as possible.

When possible, the answers to questions about similar issues were combined

so that the same coding scheme could be used for multiple questions. Namely,

school, teacher and principal were coded together, mother and father were coded

together, and all the responses to problem situations were coded together. The

coding of interview responses is listed in **Table 11**.

Table 11: Coding of interview responses for content

Question and coding category	Examples of utterances
What happens at school? What does a teacher/ principal do?	
Violence or punishment	""Sometimes the big children kick the small children. And they fight.."
Education	"She teach we."
Routine	"He come in the school yard and talk at the mike [microphone]."
Fun	"We dance and have sports."
Other	Anything else
What does a mother/father do?	
Violence or punishment	"S/he beat we." "Cuss. And beat."
Housework and child care	"She cooks dinner, wash the clothes, clean the house, wash the dishes." "He take care of the children."
Employment and financial	"S/he work." "Him give my mother money."
Education	"She send we to school." "My father, he have to see that I do my homework."
Fun and love	"Sometimes he carry me out. Sometimes he carry me to the beach." "He'll play with us." "My father likes me."
Other	"He smoke cigarette, and he always buy beer and he always sleep." "She go to church." "Mother go to dance."
What does a policeman do?	
Violence or punishment	"He shoot people." "Fire the gun." "Catch criminal and nail them to the wall." "He lock them up in jail."
Protective	"Stop war/murderer." "He help you to cross the street." "He take away people guns." "Protect you from the gunmen."
Routines	"He works in his office." "They drive jeeps."
Other	"Curse the world."
What happens when...?	
Violence or punishment	"I get beaten."
Passive situation assessment	"Can't get lunch." "Won't have book." "I will miss my class and it will take long."
Immediate response (emotional, physiological or physical action)	"I'll be sad/ hungry/ mad." "I'll cry/ bawl/ suffer."
Active problem solving (positive and negative)	"When I go to school, I ask teacher if I can order lunch and the next day pay for it." "I look for it." "Sometimes you might fight children over their lunch." "Steal."
Tell "grown-up"	"Tell the teacher/ principal" "Tell my mother/ father."
Interactions or reactions from parents, friends, teachers	"My mother going to ask me why I come so late." "The teacher will talk with them."
Miscellaneous	"I'm never late for school." "They don't tief [steal] my money"

Statistical Analysis

Three major sets of analyses were used for the Jamaican data: Study 1 alone, Study 2 alone, Study 1 and 2 together. Most of the description in this section will revolve around the analyses utilized for the studies individually, although there is a brief description of the combined analysis at the end. More details about the techniques used for both studies together will be described in the results.

Sample size

Required sample sizes were calculated using data obtained in pilot experiments and were adequate to detect differences greater than 0.5 S.D between experimental and control groups for the major outcome measures (cortisol, heart rate, catecholamines, and behaviour ratings) as statistically significant at the 0.05 level with a power of 90% (Armitage and Berry, 1994). All statistical analyses were two-tailed and performed using a commercial computer program (SPSS 7.1 for Windows: Chicago, IL). Height for age z-scores of the NCHS references were calculated using EpiNut (CDIC/WHO).

Behavioural ratings

Mean behavioural rating values were created for the interview from the two ratings (beginning and end of test) and for the frustrating tasks from the ratings taken in each individual activity (beads and Etch-a-Sketch). Since movement was a bi-modal rating, it was recoded and condensed into two 3-point ratings. Differences between the stunted and non-stunted groups in behaviours were then evaluated using t-tests and 2-way analyses of variance with sex as a second factor when the behaviour differed significantly by sex (inhibition and happy affect ratings).

Repeated measures analyses of variance over time were used to examine group differences in responsive vocalizations (2 analyses of 6 measurements each).

After these initial analyses, covariates were entered into the calculations in order to control for social background.

Salivary cortisol

The distribution of salivary cortisol was positively skewed, so the data were log transformed, and were subsequently normally distributed. The transformed data were used in all analyses. When the raw data are presented, medians and interquartile ranges are used. However, when statistical analyses are described, logged values or geometric mean values are reported.

Repeated measures analyses of variance were used to examine group differences in cortisol over time (3 measurements). For some analyses, mean cortisol was used. The value for "mean cortisol" was obtained by taking the mean of the three cortisol points. In the rare case where a child had values for only two of the three points, the two remaining values were combined as the mean.

Heart rate

Mean heart rate was calculated for each test event, and for the periods between each event (total of 20 events), and mean heart rates for each event were normally distributed. Repeated measures analyses were used to evaluate group differences over time (16 measurements) during the psychological stress session (from sitting baseline through to the final frustrating task). Linear regression was used to assess the effects of postural change

The value for "mean heart rate during psychological testing" was obtained by multiplying the mean values for each event by the duration of the particular event, and dividing by total time. This measure was used to isolate one variable that represented cardiac arousal due to psychological testing.

Urinary catecholamines

One measurement, expressed per volume of creatinine, was obtained of each urinary catecholamine, epinephrine, norepinephrine and dopamine. Dopamine and norepinephrine concentrations were normally distributed, but epinephrine levels were positively skewed; epinephrine levels were log transformed and subsequently normal. Differences between the stunted and non-stunted groups in urinary catecholamines were evaluated using 2-way analyses of variance and then repeated including covariates.

Interactions between physiology and behaviour

To investigate the relationship between cortisol and behaviour, a linear regression was calculated with both groups together, behaviour as the dependent variable, and group, baseline cortisol, and group x cortisol as the independent variables.

Socio-economic considerations

Bivariate correlations were calculated between each dependent variable and all socio-economic variables, height for age, birthweight, and IQ's of mother and child with groups separate, and there were no significant associations. However, due to the small sample sizes, all analyses of group differences using as covariates socio-economic status (economic and stimulation indices), and maternal and child IQ. After showing that none of the covariates contributed significantly to any analysis, and to exclude the possible role of birthweight, the analyses of cortisol, heart rate and epinephrine, group differences were repeated with birthweight squared as the only covariate. Birthweight has been shown to be quadratically related to cortisol (Clark et al. 1996), and the stunted group in this study had slightly smaller birth weights.

Interview results

A composite "aggression" variable was calculated by coding each of the twelve interview responses for each child with a 0 or a 1. In order for a response to be coded 1, it had to include one of the following words in the context of describing violence or aggression: fight, beat, kick, kill, shoot, lick, fire (gun), hit, stab, cut, throw, war, gunshot, burst (bust), or refer to excessive corporal punishment or bullying. The codes were then summed, giving the "aggression rating" a potential range of 0-12, with an actual maximum of 10, mean of 3.92, and standard deviation of 2.38.

A linear regression was performed, with "aggression vocalization" as the dependent variable. Entered in one step as independent variables were: age, sex, all socio-economic variables (maternal IQ, subject IQ, economic rating, stimulation rating, birthweight), anthropometric variables (height for age, BMI), and physiological variables (norepinephrine, epinephrine, dopamine, mean cortisol, mean heart rate, baseline heart rate).

Analysis with groups combined

The analyses above describe the techniques used when analyzing Study 1 and Study 2 independently. In order to incorporate all four groups into one analysis, a regression was used, predicting physiological outcome from group (coded as a dummy variable) and several covariates. This analysis is described in further detail in the Results (Chapter 8).

Chapter 7: Results from physiological and behavioural testing of Jamaican children

Introduction

The results will be presented in three sub-sections: results from Study 1, results from Study 2 and then results from both studies combined. Summaries of the data will be presented at the beginning of each of the results sub-sections, and then analyses will be described. Shown in **Table 12** is the length of each event in the testing session.

Table 12: Mean and standard deviation (SD) of duration of events in the testing session [1]

Event	Duration (min)
Arrival[2]	
Baseline heart rate (lying)	13.2 (3.7)
Postural Change	1.2 (2.2)
Baseline heart rate (sitting)	11.4 (3.6)
Interview	11.0 (4.7)
Mental arithmetic	3.6 (1.0)
Symbol Digit Modalities test	2.1 (0.8)
Pegboard test	2.2 (1.4)
Frustrating tasks	13.1 (2.7)
Isometric handgrip task	2.0 (0.2)
Baseline heart rate (lying)[2]	16.5 (5.1)
Departure	

[1] There were approximately 3 minutes between events.

[2] Time between arrival and baseline (lying) was variable (range 5-60 minutes).

Results from Study 1

Sample Characteristics

        Characteristics of the sample are described in **Table 13**. Besides the

expected differences between groups in height-for-age, significant differences

between the stunted and non-stunted groups are also evident for birthweight, body

mass index, subject and maternal PPVT, and the economic and stimulation indices.

Table 13: Mean and standard deviation (SD) of characteristics of the stunted and non-stunted children in study 1

Characteristic[1]	Stunted (n = 30)	Non-stunted (n = 24)	Difference (95% CI)	P[2]
Age (m)	112.7 (5.0)	111.7 (5.2)	1.1 (-1.7, 3.9)	NS
Birthweight (kg)	2.9 (0.4)	3.5 (0.6)	-0.6 (-0.9, -0.3)	<0.0001
Height for age (z-score)	-1.7 (0.5)	1.0 (0.7)	-2.7 (-3.0, -2.3)	<0.0001
Body Mass Index (BMI, kg/m^2)	14.6 (1.3)	16.2 (1.7)	-1.6 (-2.5, -0.8)	<0.0001
Economic index	-0.6 (1.6)	0.8 (1.5)	-1.5 (-2.4, -0.6)	0.001
Stimulation index	5.8 (3.7)	8.2 (2.5)	-2.4 (-4.2, -0.6)	0.009
Child PPVT (raw score)	38.4 (14.8)	47.0 (9.8)	-8.7 (-15.7, -1.6)	0.02
Maternal PPVT (raw score)	78.4 (19.5)	98.2 (19.1)	-19.9 (-30.5, -9.22)	<0.0001
Sex (M/F)	18/12	13/11		NS

[1] Characteristics are listed as assessed at the time of testing, except birthweight, the economic and stimulation indices, and maternal PPVT, all of which were assessed during the home interview. Subject PPVT was assessed 6 months prior to testing.
[2] P values were calculated by the Pearson chi-square test for sex, and by the two-sample Student's t-test for all other variables. NS = non-significant

Behavioural measures

As shown in **Table 14**, the stunted and non-stunted children differed for several of the behaviours that were measured, particularly the inhibition and happy ratings. It is important to note that the Inhibition rating is coded so that a low value represents a child who is very inhibited whereas a high rating represents an outgoing child. For behaviours measured multiple times during a frustrating task (vocalizations, strategies used, number of times shake Etch-a-Sketch), only the values for the first half of the event are reported. In this way there is no bias towards children who stopped half way through, or those who continued with the project for the whole time period.

Table 14: Means and standard deviations of behavioural observations for stunted and non-stunted children in Study 1

Behaviour	Stunted (n = 30)	Non-stunted (n = 24)	Mean difference (CI)	P[1]
Interview:[2]				
Vocalization, anwers to "non-troubling" questions	2.9 (0.9)	3.3 (0.8)	-0.4 (-0.8, 0.1)	NS
Vocalization, answers to "troubling" questions.	2.9 (0.9)	3.4 (0.7)	-0.5 (-0.9, 0.03)	0.03
Inhibition rating[3]	2.5 (1.3)	3.3 (1.3)	-0.7 (-1.5, -.02)	0.03
Happy Affect rating	3.0 (1.1)	3.6 (1.1)	-0.5 (-1.2, 0.1)	0.08
Frustrating tasks:				
Spontaneous vocalizations	6.7 (8.7)	7.5 (6.1)	-0.5 (-2.7, 1.7)	NS
Frustration	3.6 (1.0)	3.3 (1.0)	0.3 (-0.2, 0.9)	NS
Attention	3.6 (0.9)	4.1 (0.7)	-0.6 (-1.0, -0.08)	0.02
Happy Affect	3.0 (0.9)	3.2 (0.9)	-0.2 (-0.7, 0.2)	NS
Strategies used in beads	3.6 (1.2)	4.0 (0.9)	-0.4 (-1.0, 0.2)	NS
No. times shake Etch-a-sketch (persistence)	3.6 (3.3)	5.1 (4.2)	-1.6 (-3.7, 0.5)	NS

[1] P values were calculated by the two-sample Student's t-test for all variables.
[2] Movement rating was excluded because it was not a linear rating
[3] Inhibition rating ranges from 1=very inhibited, 5=very outgoing

To avoid the errors generated by over-use of statistical tests, the ratings were analyzed with repeated measures ANOVA's or with mean ratings whenever possible.

Differences between the groups in responsive vocalization during the interview were examined using two repeated measures analyses of variance, first of the six troubling questions, and then of the six non-troubling questions. Stunted children had a tendency to talk less, and this difference ($\pm$ S.D.) was significant for the six troubling questions (mean rating, 2.9 ± 0.9 vs. 3.4 ± 0.7, P=0.03), but not for the non-troubling ones (mean rating, 2.9 ± 0.9 vs. 3.3 ± 0.8, P=0.15). These findings did not withstand inclusion of the five SES variables, however the group differences did remain significant with the inclusion of birthweight.

Stunted children were more inhibited in the interview (mean rating, 2.5 ± 1.3 v. 3.3 ± 1.3, P=0.03) and less attentive in the frustrating task (mean rating, 3.6 ± 0.9 vs. 4.1 ± 0.7, P=0.02) than non-stunted children. Both of these significant group differences were sustained when the SES and IQ variables were used as covariates. When birthweight alone was used as a covariate, all group differences remained.

Although inhibition and attention were the only significant ratings where the groups were significantly different, there is a clear pattern of behaviour that emerges, with stunted children more frustrated and less happy than non-stunted children. Stunted children also had a tendency to utilize fewer strategies when presented with the bead task, and to shake the Etch-a-Sketch (thereby clearing the drawing surface, and beginning on a new drawing attempt) less frequently than the non-stunted children, suggesting that they were less persistent than non-stunted children. No differences were evident between groups in terms of the types of vocalizations made during the frustrating task, or in the types of movement made in either task.

Salivary cortisol

Stunted children had significantly higher salivary cortisol levels than non-stunted children when compared using repeated measures analysis of variance over the three time points (group effect, $F(1,48)=8.0$, $P=0.007$, test effect, $F(2,96)=15.7$, $P<0.001$). Since salivary cortisol concentrations are positively skewed, they are represented with median and inter-quartile range in **Table 15**, although statistical analyses were completed using log-transformed values. Cortisol concentrations in both groups declined over the test period and there was no significant interaction between group and test (**Figure 10**).

Table 15: *Median and inter-quartile range of salivary cortisol concentrations (µg/dL) in stunted and non-stunted children from Study 1.*

Timing of sample	Stunted (n = 29)	Non-stunted (n = 24)[1]
	µg/dL	
Baseline	0.22 (0.21)	0.15 (0.17)
Mid-test	0.17 (0.20)	0.14 (0.12)
Post-test	0.15 (0.12)	0.12 (0.10)

[1] n = 25 for Mid-test point

The group difference between stunted and non-stunted children from Study 1 remained significant when the four SES and IQ covariates were entered into the analysis ($F(1,44)=5.2$, $P=0.03$), and none of the covariates was significant. When birthweight was used as a covariate, it did not affect the findings ($F(1,47)=5.9$, $P=0.02$), and birthweight was not significant.

3

2

1

0

Salivary Cortisol (μg/L)

Baseline   Mid-test   Post-test
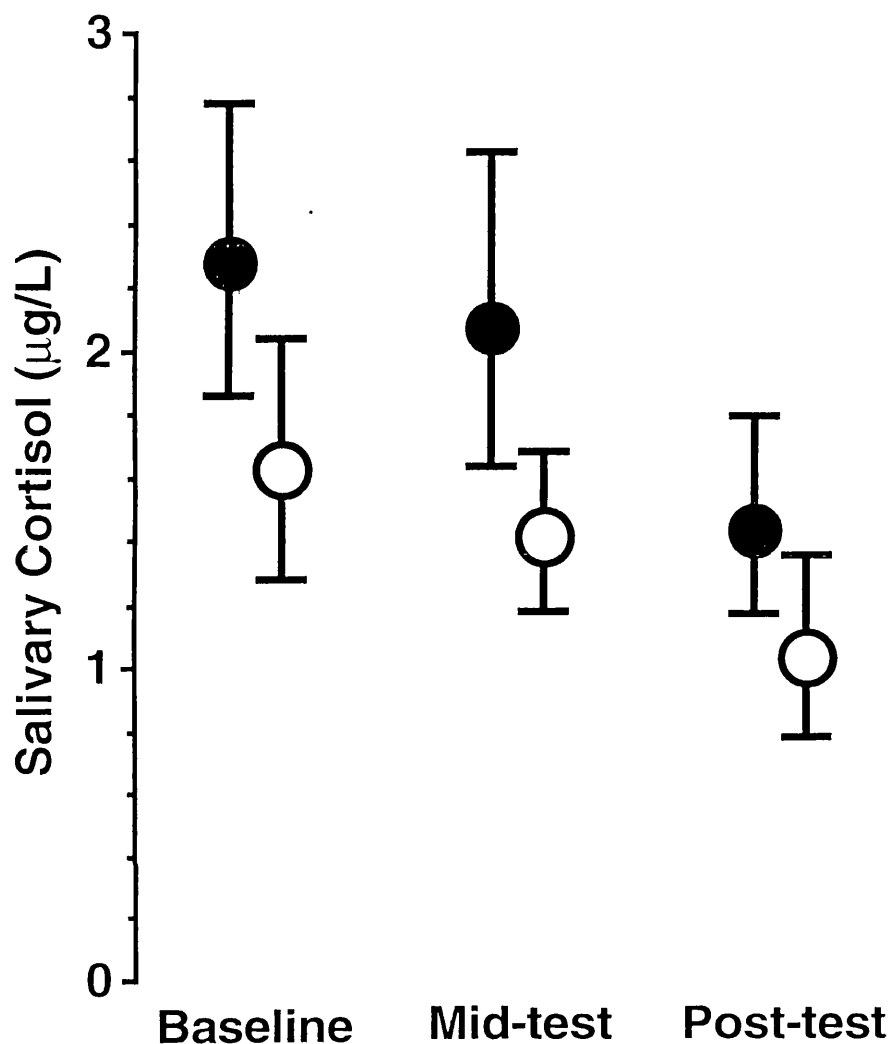
Figure 10: Salivary cortisol levels during the test session, according to study group

Geometric mean salivary cortisol concentrations during the test session in stunted (s; $n = 28$ for baseline and $n = 29$ for mid- and posttest) and nonstunted (d; $n = 23$ for posttest and $n = 24$ for baseline and midtest) children. Bars represent 95% CIs. Filled circles represent stunted children and empty circles represent non-stunted children.

Baseline cortisol had a negative effect on vocalizations in both the interview (B=-0.55, P=0.11) and frustrating tasks (B=-0.45, P=0.37) in the stunted children, and positively correlated in the interview (B=0.58, P=0.02) and the frustrating tasks (B=1.42, P=0.004) in the non-stunted children (**Figure 11**) with a significant

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interaction (responsive vocalizations, P=0.009, spontaneous vocalizations, P=0.007) (**Table 16**). Analogously, high levels of salivary cortisol were associated with inhibition in stunted children (B=-1.80, P=0.04) and with outgoing behaviour in non-stunted (B=1.01, P=0.29) children, with a significant interaction (P=0.03) (**Figure 11**). These interactions remained highly significant after the inclusion of all covariates.

Table 16: Regression coefficient and 95% confidence intervals for effect of cortisol on behaviour in stunted and non-stunted children in Study 1

Behaviour	Stunted (n = 29)	Non-stunted (n = 24)
Responsive vocalizations	-0.55 (-1.23, 0.13) [1]	0.58 (0.08, 1.08) [2]
Spontaneous vocalizations	-0.45 (-1.47, 0.56)	1.42 (0.51, 2.34) [3]
Inhibition rating [4]	-1.80 (-3.53, -0.07) [2]	1.01 (-0.91, 2.94)

[1] Values for the B, the regression coefficient were calculated in a linear regression with groups separately, with the behaviour as the dependent variable, and the baseline salivary cortisol level as the independent variable.

[2] P<0.05 for comparison between the stunted and non-stunted groups.

[3] P=0.004 for comparison between the stunted and non-stunted groups.

[4] The inhibition rating ranges from 1 = very inhibited and 5 = very outgoing.
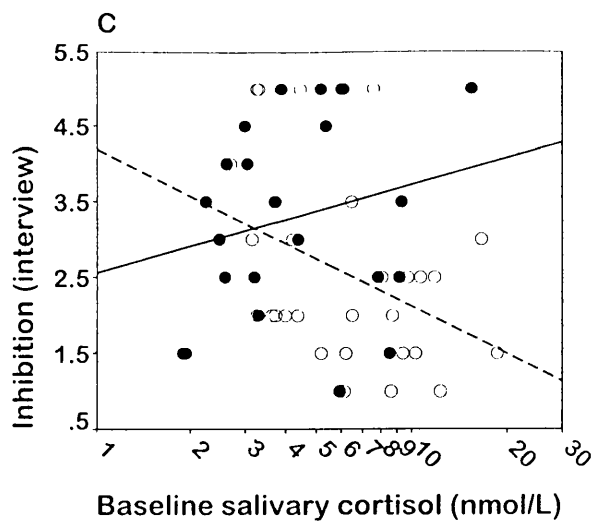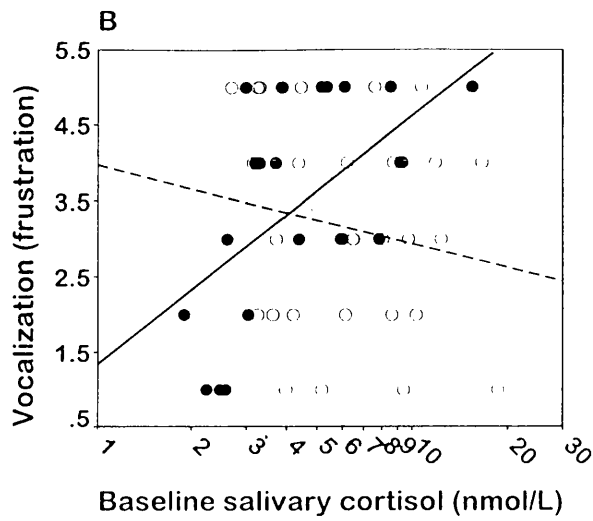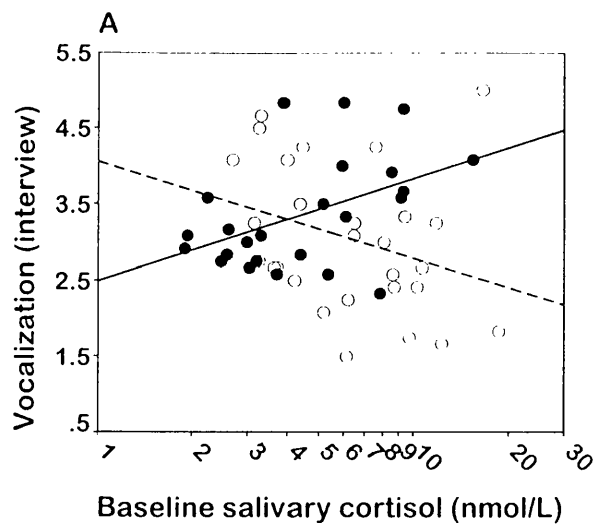
Figure 11: *Relationship between baseline salivary cortisol level (geometric mean) and interview inhibition rating (A), vocalization rating during interview (B), and vocalization during frustrating tasks (C)*

Dashed lines represent stunted children and solid lines represent non-stunted children.

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Heart rate

Both groups had similar overall response patterns of heart rate change over time, and reached their maximum heart rates during the same testing event, but stunted children had higher heart rates at every point in the testing sequence than non-stunted children (**Table 17**).

Table 17: Mean and standard deviation (S.D.) heart rates for stunted and non-stunted subjects in Study 1.

	Stunted (n = 30)[1]	Non-stunted (n = 24)[1]
	beats/min	
Baseline (lying)	80.6 (5.8)	77.2 (9.6)
Physiological stressor (postural change)	92.4 (8.4)	83.7 $(8.5)^2$
Baseline (sitting)	88.8 (8.7)	84.9 (9.5)
Psychological stressors	93.8 (6.3)	89.0 $(8.0)^2$
Baseline (lying)	85.0 (6.4)	79.2 $(11.6)^2$

[1] n = 21 for physiological stressor in both groups
[2] P<0.05 for comparison between the stunted and non-stunted groups.

Initial lying baseline heart rate were not significantly different (80.6 ± 5.8 v. 77.2 ± 9.6), although the final lying baseline heart rates were (85.0 ± 6.4 v. 79.2 ± 11.6, P=0.03) (**Figure 12**). The group difference for the final baseline was of borderline significance when SES and IQ covariates were included (P=0.05), although none of the covariates was significant. When birthweight was used as a covariate, the group difference at final lying baseline remained significant (P=0.04).
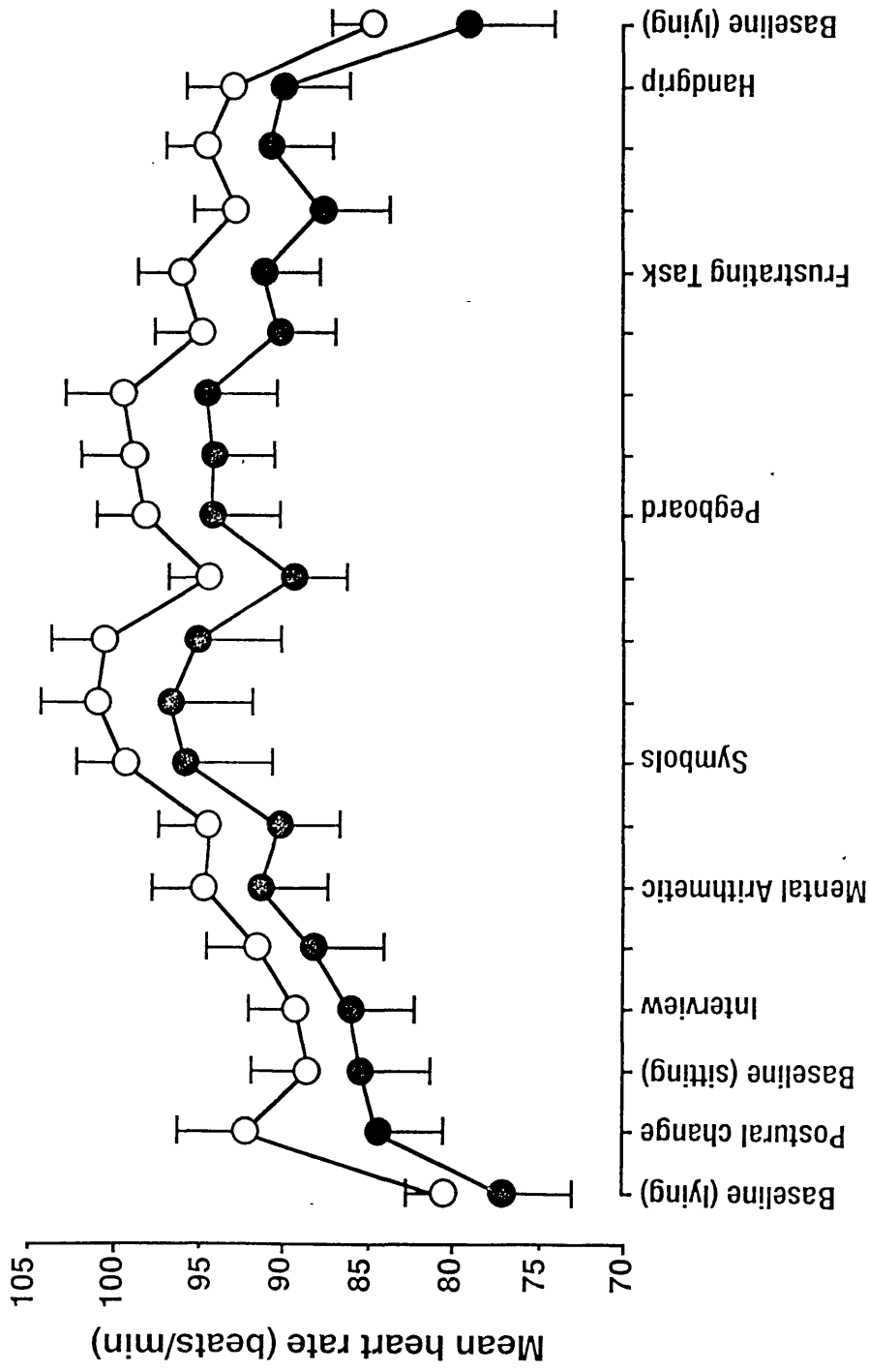
Figure 12: Heart rate during test session, according to study group in Study 1.

Empty circles represent stunted children (n=30 for all tests except postural change where n=21), and filled circles represent non-stunted children (n=23 for all tests except postural change where n=20). The lines show the 95 percent confidence intervals for the means in each group at each point. Both the symbols and the pegboard tests were repeated twice.

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The effect of postural change was evaluated in a linear regression with mean heart rate during postural change as the dependent variable, and lying baseline and group (stunted/non-stunted) as independent variables. This analysis allowed for the evaluation of actual heart rate responsivity while controlling for baseline level. There was a significant group effect (B=-4.4, P=0.04), with no change in significance after adding any of the covariates as additional independent variables.

In repeated measures analysis of variance over the psychological test session (16 test points) starting from the sitting baseline heart rate, there was a significant group effect (F(1,51)=5.0, P=0.03) and test effect (P<0.0001). The group effect no longer reached significance when the SES and IQ (F(1,47)=3.3, P=0.07) or birthweight covariates (F(1,50)=3.6, P=0.06) were added. No cardiovascular response to the isometric hand grip was evident in either group.

The mean heart rate was not significantly associated with behaviour ratings in either group. The mean heart rate was significantly associated with mid-test (r=0.33, P=0.03) and post-test cortisol levels (r=0.43, P=0.02) in stunted children. None of the correlations in the non-stunted group reached significant levels, but the interactions were not significant.

Urinary catecholamines

Stunted children had higher levels of epinephrine than non-stunted children (**Table 18**), and the differences were significant (geometric mean: 0.013 v. 0.008, group effect $F(1,52)=11.9$, $P=0.001$). There were no significant differences between groups for levels of urinary norepinephrine or dopamine.

Table 18: Overview of results for urinary catecholamine analysis (dopamine, epinephrine, norepinephrine) by group for Study 1. Mean and standard deviation (SD) presented for dopamine and norepinephrine. Median and interquartile range (IQR) presented for epinephrine.

Catecholamine	Stunted (n = 30)[1]	Non-stunted (n = 24)[2]
	μmol/mmol creatinine	
Dopamine	0.396 (0.102)	0.373 (0.109)
Epinephrine	0.013 (0.006)	0.008 (0.006)*
Norepinephrine	0.027 (0.008)	0.028 (0.013)

[1] n = 29 for epinephrine
[2] *, P<0.05, **, P<0.01

After inclusion of the socio-economic covariates into the analysis, the results still approach significance (group effect $F(1,48)=2.0$, $P=0.16$), and none of the covariates are significant. The effects remain strongly significant when the analysis is repeated with the inclusion of birthweight for epinephrine (group effect $F(1,51)=14.0$, $P<0.0001$).

Results from Study 2

Sample characteristics

Characteristics of the sample are described in **Table 19**. The groups were not different in terms of age, economic or stimulation indices, or maternal IQ. However, the stunted children were thinner, had lower PPVT's, and had lower birth weights than the non-stunted children.

Table 19: Mean and standard deviation (SD) of characteristics of the stunted and non-stunted children in Study 2

Characteristic[1]	Stunted (n = 31)[3]	Non-stunted (n = 31)	Difference (95% C.I.)	(P)[2]
Age (m)	112.6 (6.8)	112.7 (7.1)	-0.2 (-3.7, 3.3)	NS
Birthweight (kg)	3.1 (0.4)	3.4 (0.6)	-0.3 (-0.6, -0.006)	0.046
Height for age (z-score)	-2.3 (0.4)	0.4 (0.8)	-2.4 (-2.7, -2.1)	<0.0001
Body Mass Index (BMI, kg/m^2)	14.9 (1.3)	15.9 (2.1)	-1.0 (-1.9, -0.1)	0.02
Economic index	0.4 (1.4)	0.3 (1.8)	0.7 (-0.2, 1.5)	NS
Stimulation index	6.5 (2.5)	5.6 (3.6)	0.8 (-0.7, 2.4)	NS
Subject PPVT (raw score)	49.5 (11.0)	56.2 (14.1)	-6.7 (-13.1, -0.3)	0.04
Maternal PPVT (raw score)	85.3 (25.0)	92.5 (25.0)	-7.2 (-20.1, 5.7)	NS
Sex (M/F)	21/10	21/10		NS

[1] Characteristics are listed as assessed at time of testing, except birthweight, the economic and stimulation indices, and maternal IQ (assessed during the home interview).
[2] P values were calculated by the Pearson chi-square test for sex, and by the two-sample Student's t-test for all other variables. NS = non-significant
[3] Birthweight, n = 25 stunted, n = 23 nonstunted; maternal PPVT, n = 31 stunted, n = 29 nonstunted

Behavioural measures

Table 20: *Mean and standard deviation (SD) of behavioural observations for stunted and non-stunted children in Study 2.*

Behaviour	Stunted (n = 31)	Non-stunted (n = 31)	Mean difference (C.I.)	P[1]
Interview[2]:				
Vocalization, answers to "non-troubling" questions	3.0 (0.8)	3.4 (0.9)	-0.4 (-0.8, 0.06)	0.09
Vocalization, answers to "troubling" questions	3.0 (0.8)	3.4 (0.9)	-0.4 (-0.8, 0.03)	0.07
Inhibition rating[3]	2.5 (1.3)	3.5 (1.2)	-1.0 (-1.6, -0.3)	0.003
Happy Affect rating	2.6 (1.1)	3.5 (0.7)	-0.9 (-1.3, -0.4)	<0.0001
Frustrating tasks:				
Spontaneous vocalizations	6.4 (6.9)	6.1 (5.5)	0.8 (-1.1. 1.4)	NS
Frustration	3.8 (1.0)	2.9 (1.2)	0.9 (0.3, 1.5)	0.002
Attention	3.6 (1.1)	4.0 (0.9)	1.8 (-0.8, 0.2)	NS
Happy Affect	2.8 (0.9)	3.2 (0.8)	-0.4 (-0.8, 0.05)	0.08
Strategies used	3.6 (1.5)	4.2 (1.1)	-0.6 (-1.3, 0.1)	0.08
No. times shake Etch-a-sketch (measuring persistence)	3.5 (2.0)	4.5 (2.1)	-1.0 (-2.1, 0.06)	0.06

[1] P values were calculated by the two-sample Student's t-test for all other variables.
[2] Movement rating was excluded because it was not a linear rating
[3] Inhibition rating, 1=very inhibited, 5=very outgoing

As shown in **Table 20**, stunted children differed from non-stunted children in several of the observed behaviours. Stunted children were more inhibited in the interview (mean rating, 2.5 ± 1.3 v. 3.5 ± 1.2, P=0.003), less happy in the interview (mean rating, 2.6 ± 1.1 v. 3.5 ± 0.7, P<0.0001), and more frustrated in the frustrating tasks (mean rating, 3.8 ± 1.0 v. 2.9 ± 1.2, P=0.002). All differences (inhibition, happy, frustration) remained highly significant (P<0.01) with the inclusion of the four SES covariates, and inhibition and happy affect remained significant (P<0.01) with the inclusion of birthweight. Frustration bordered on significant (P=0.07) with

the inclusion of birthweight, although birthweight did not come in to the analysis. The loss of significance is probably due to the substantial loss of power due to the small number of children within the sample who had birthweight data.

Differences between the groups in responsive vocalization during the interview were assessed with two repeated measures analyses of variance, first of the six troubling questions, and then of the six non-troubling ones. Stunted children talked less than the non-stunted children, although this difference was not significant for either the six troubling questions (3.0 ± 0.8 v. 3.4 ± 0.9, P=0.07) or the six non-troubling ones (3.0 ± 0.8 v. 3.4 ± 0.9, P=0.09).

Stunted children also had a tendency to be less happy during the frustrating task (2.8 ± 0.9 v. 3.2 ± 0.8, P=0.08) than non-stunted children. They used fewer strategies when trying to figure out how to thread the beads (mean number of strategies in first half, 3.6 ± 1.5 v. 4.2 ± 1.1, P=0.08) and were less persistent when trying to draw a circle on the Etch-a-Sketch (number of re-starts, 3.5 ± 2.0 v. 4.5 ± 2.1, P=0.06).

Salivary cortisol

Stunted children did not differ from non-stunted children in their salivary cortisol concentrations, when compared using repeated measures analysis of variance over the three time points (group effect, $F_{(1,52)}=1.7$, $P=0.19$), although there was a significant test effect ($F_{(2,106)}=8.2$, $P<0.0001$). Cortisol concentrations declined over the test period in stunted children, and peaked at the mid-test point for the non-stunted children, suggesting that the groups may have been different in their reactivity. However, in a regression predicting the mid-test point from baseline and group, there was neither a group effect or a group x test interaction. The data for the three time points are presented in **Table 21**.

Table 21: Median and interquartile range for salivary cortisol concentrations (µg/dL) in stunted and non-stunted children from Study 2.

Timing of sample	Stunted (n = 30)[1]	Non-stunted (n = 30)[2]
	µg/dL	
Baseline	0.31 (0.32)	0.28 (0.18)
Mid-test	0.30 (0.21)	0.34 (0.23)
Post-test	0.22 (0.20)	0.20 (0.16)

[1] n = 29 for Post-test
[2] n = 31 for Baseline point

There were no significant correlations between cortisol levels and interview vocalizations or any other behaviours.

Heart rate

Stunted children had higher heart rates than non-stunted children at every point in the testing sequence than non-stunted children, and both groups had very similar overall response patterns of heart rate change over time. As shown in **Table 22**, stunted children had higher initial lying baseline heart rates than non-stunted

children (90.8 ± 10.8 v. 83.4 ± 8.8, P=0.01), and higher initial sitting baseline heart rates in addition (94.7 ± 10.6 v. 89.3 ± 9.6, P=0.04). The stunted children also had higher final lying baselines, but these differences did not reach significance (88.0 ± 12.6 v. 82.7 ± 8.1, P=0.06).

Table 22: Mean and standard deviation (S.D.) heart rates for stunted and non-stunted subjects in Study 2

	Stunted (n = 31)[1]	Non-stunted (n = 31)
	beats/min	
Baseline (lying)	90.8 (10.8)	83.4 (8.8)[2]
Physiological stressor (postural change)	97.5 (10.4)	93.1 (8.5)
Baseline (sitting)	94.7 (10.6)	89.3 (9.6)[2]
Psychological stressors	98.0 (9.1)	92.1 (8.9)[2]
Baseline (lying)	88.0 (12.6)	82.7 (8.1)

[1] n = 29 for Physiological stressor
[2] P<0.05 for comparison between stunted and non-stunted groups

The differences between groups for the first lying baseline remained significant with the inclusion of the four socio-economic variable (P=0.02), and the inclusion of birthweight (P=0.03). The difference between groups at the sitting baseline lost significance with inclusion of SES variables (P=0.07) and birthweight (P=0.05).

Repeated measures analysis of variance over the psychological test session showed a group difference that approached significance ($F(1,60)=3.7$, P=0.06) and a significant test effect (P<0.0001). The significance of the group effect dropped with the inclusion of SES and birthweight variables (P=0.1). No cardiovascular response to the isometric hand grip was evident in either group.

The "mean psychological heart rate," described in Methods, was not significantly associated with behaviour ratings in either group, but was significantly correlated with the mean cortisol level in non-stunted children (r=0.39, P=0.03).

Urinary catecholamines

As shown in **Table 23**, stunted children had higher levels of epinephrine than non-stunted children and the differences were significant (geometric mean: 0.017 v. 0.012, group effect $F(1,59)=7.4$, $P=0.009$). Stunted children also had signifantly higher concentrations of norpeinephrine (0.29 v. 0.24, group effect $F(1,59)=6.2$, $P=0.016$). There were no significant differences between groups for levels of dopamine.

Table 23: Urinary catecholamines (dopamine, epinephrine, norepinephrine) in stunted and non-stunted children from Study 2. Mean and standard deviation (SD) presented for dopamine and norepinephrine. Median and interquartile range (IQR) presented for epinephrine.

Catecholamine	Stunted (n =31)	Non-stunted (n = 30) [1]
	μmol/mmol creatinine	
Dopamine	0.425 (0.119)	0.412 (0.377)
Epinephrine	0.018 (0.01)	0.013 (0.007)**
Norepinephrine	0.029 (0.007)	0.024 (0.007)*

[1] * P<0.05, ** P<0.01

After inclusion of the socio-economic covariates into the analysis, the results were still significant for epinephrine (group effect $F(1,53)=6.0$, $P=0.018$), and approaching significance for norepinephrine (group effect $F(1,53)=3.8$, $P=0.057$). In both of these analyses, no covariates came into the model. When the analysis was repeated including birthweight as the only covariate, the group differences were

highly significant for epinephrine (group effect $F_{(1,58)}=6.1$, $P=0.016$) and

norepinephrine (group effect $F_{(1,58)}=5.1$, $P=0.027$).

Results with all Jamaican groups combined

Introduction

The samples in Study 2 were intended to enlarge those in Study 1, and so the initial intent was to combine the two stunted groups and the two non-stunted groups, thereby gaining the statistical power lost by having such small samples from the longitudinal follow-up (Study 1). However, in comparing the four groups individually, it was clear that they were not only different in terms of their nutritional status because the stunted group that had been followed longitudinally was significantly taller than the newly recruited group. The groups also differed substantially in terms of their testing experience. Given the effects of novelty on stress response, the difference in experience (i.e. how many times the child has been up the the University for testing) was significant. Thus, all analyses of Studies 1 and 2 together retained four separate "groups."

A multiple regression was completed predicting the mean physiological measure (mean cortisol, mean heart rate for the psychological testing), with age and sex entered on the first step, four covariates offered on the second step (body mass index, maternal PPVT, stimulation rating, economic rating), and group (three dummy variables) entered on the third step. This analysis basically quantified the difference between stunted and non-stunted children whether they were experienced (Study 1) or inexperienced (Study 2). Thus, it allowed for the direct comparison of the effect size (B value) of stunting in two different samples on physiological and behavioural outcomes, while benefitting from the power of having a sample size larger than that from either study alone. A further benefit of this analysis is that it

allowed for the estimation of effect of testing experience in both stunted and non-stunted children.

In the first analysis, the non-stunted group from Study 1 (i.e. no stunting or novelty effects) was used as the comparison group, which gave a "stunting effect" (for children who were experienced), when it was compared with the stunted group from Study 1, and a "novelty effect" (for children who were non-stunted) when it was compared with the non-stunted group from Study 2. In the second analysis, the non-stunted group from Study 2 (i.e. no stunting effect, but novelty effect) was used as the comparison group, which gave a "stunting effect" (for the inexperienced subjects) when it was compared with the stunted group from Study 2, and an "experience effect" (for children who were non-stunted) when it was compared with the non-stunted group from Study 1. In a third analysis, the stunted group from Study 1 (i.e. stunting effect but no novelty effect) was used as the comparison group, which gave an "experience effect" (for the stunted subjects) when it was compared with the stunted group from Study 2.

Behavioural measures

Stunted children from both groups vocalized less than non-stunted children in both groups (**Figure 13**). These differences show a significant "stunting effect" in the experienced group (B=-0.49, P=0.03) and an almost significant "stunting effect" in the inexperienced group (B=-0.39, P=0.06) when age and sex were included as the only covariates. These differences lost their significance when the socio-economic covariates were offered into the regression. No experience effect was evident in either the stunted or non-stunted groups.
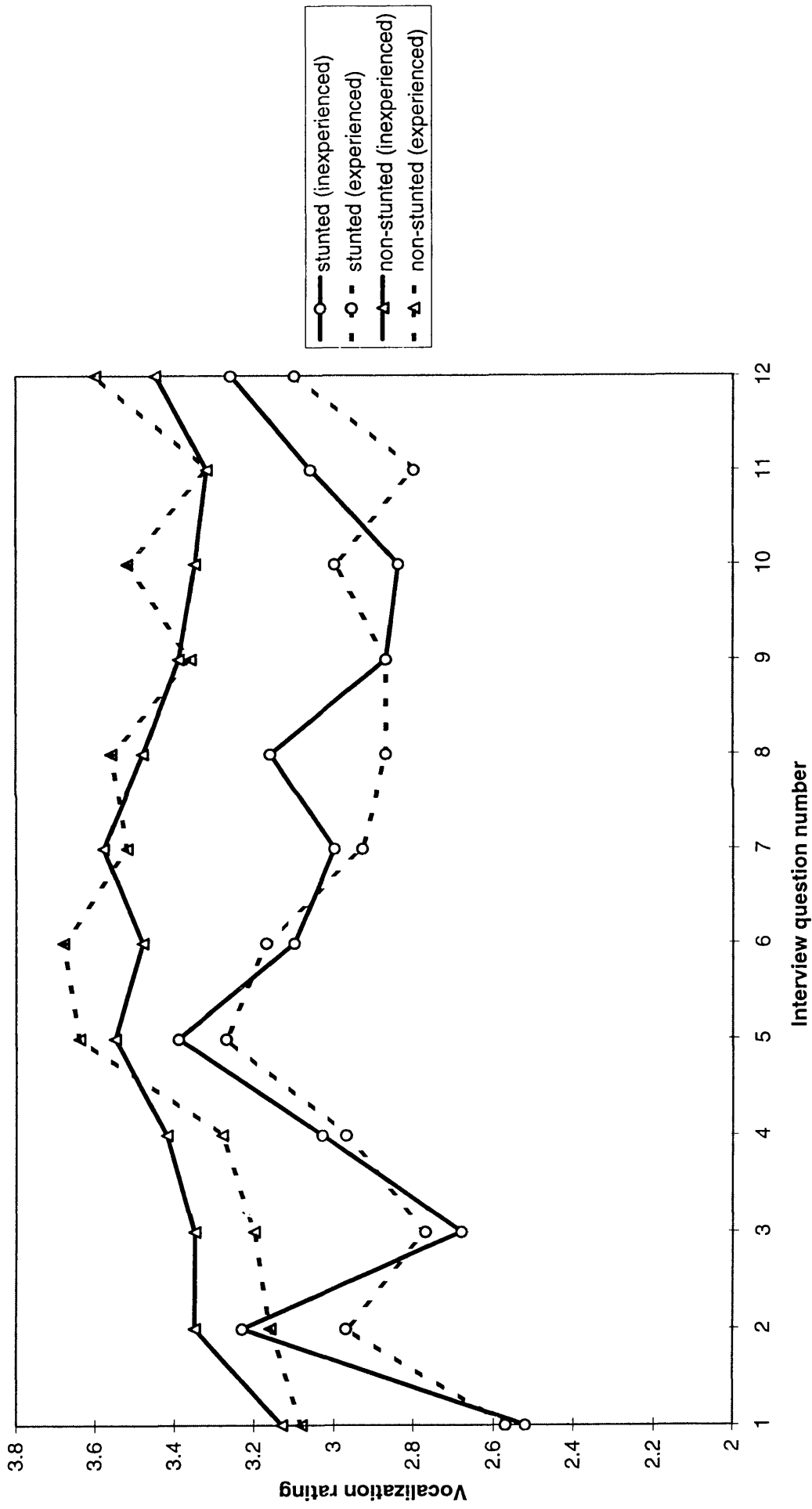
Figure 13: Interview responses for each of the interview questions for the stunted and non-stunted groups from Study 1 and Study 2.

Stunted children from both groups were more inhibited and less happy than non-stunted children in both groups (**Figure 14**). The differences between the stunted and non-stunted groups for inhibition were highly significant, and remained significant with inclusion of covariates (**Table 24**). The results show a "stunting effect" in the experienced (B=0.731, P=0.034) and the inexperienced (B=0.881, P=0.008) groups, and no "experience effect" in either group.

Table 24: *Differences between groups in a multiple regression predicting mean inhibition rating from group and several covariates (age, sex, BMI, maternal PPVT, simulation index, economic index)*

Comparison group	Variable	Mean inhibition rating	
		GROUP EFFECT	
		B value (CI)	P value
non-stunted, experienced	stunted, experienced	0.731 (0.063, 0.607)	0.034
	non-stunted, inexperienced	-0.071 (0.306, 0.835)	NS
	stunted, inexperienced	0.660 (0.547, 1.090)	0.05
non-stunted, inexperienced	stunted, inexperienced	0.881 (0.001, 0.496)	0.008
stunted, experienced	stunted, inexperienced	-0.158 (0.226, 0.741)	NS

The happy rating showed a significant "stunting effect" in the experienced group (B=-0.58, P=0.02) and a highly significant "stunting effect" in the inexperienced group (B=-0.88 , P<0.0001), with age and sex entered as covariates. These differences lost their significance when the covariates were offered into the regression, although none of the covariates were significant in the final equation. No experience effect was evident in either the stunted or non-stunted groups.
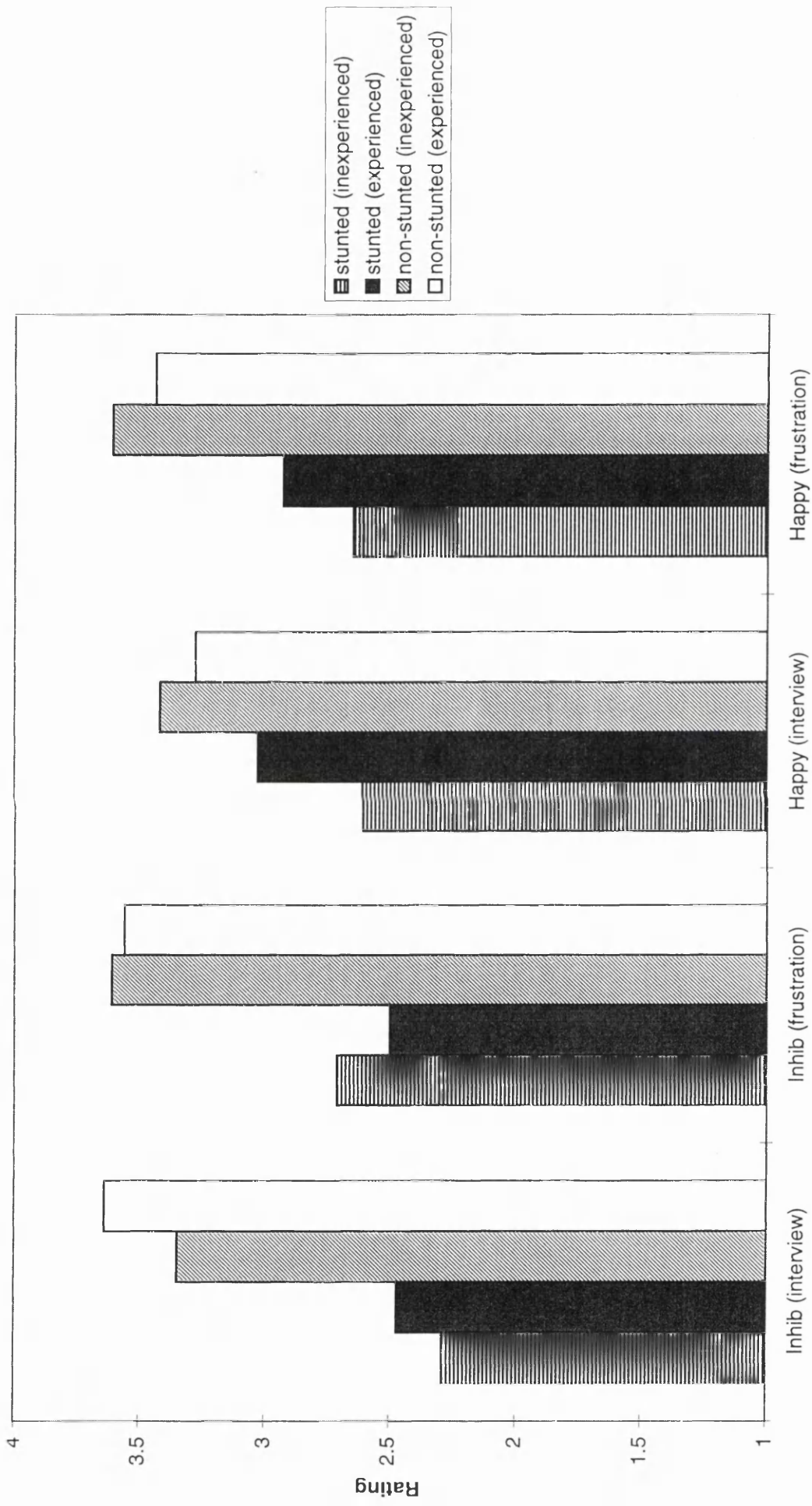
Figure 14: Behaviour ratings (happy affect and inhibition) for stunted and non-stunted children from Study 1 and Study 2.

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Salivary cortisol

Stunted children had higher concentrations of salivary cortisol than did non-stunted children, regardless of whether they had ever been tested before (**Figure 15**). When the dependent variable was mean cortisol, the "stunting effect" was evident in both the first (B=0.335, P=0.021) and the second (B=0.248, P=0.049) analyses, even when controlling for a large number of possible confounders (**Table 25**). None of the additional variables came into either analysis. A substantial "experience effect" is evident in both the stunted (B=0.438, P<0.0001) and the non-stunted (B=0.57, P<0.0001) groups, with a strong combined nutrition and experience effect (B=0.819, P<0.0001).

Table 25: Differences between groups in a multiple regression predicting log-transformed cortisol concentration from group and several covariates (age, sex, BMI, maternal PPVT, simulation index, economic index)

Comparison group	Variable	Geometric mean cortisol (mmol/L)	
		GROUP EFFECT	
		B value (95%CI)	P value
non-stunted, experienced	stunted, experienced	0.335 (0.063, 0.607)	0.016
	non-stunted, inexperienced	0.570 (0.306, 0.835)	<0.0001
	stunted, inexperienced	0.819 (0.547, 1.090)	<0.0001
non-stunted, inexperienced	stunted, inexperienced	0.248 (0.001, 0.496)	0.049
stunted, experienced	stunted, inexperienced	0.438 (0.226, 0.741)	<0.0001
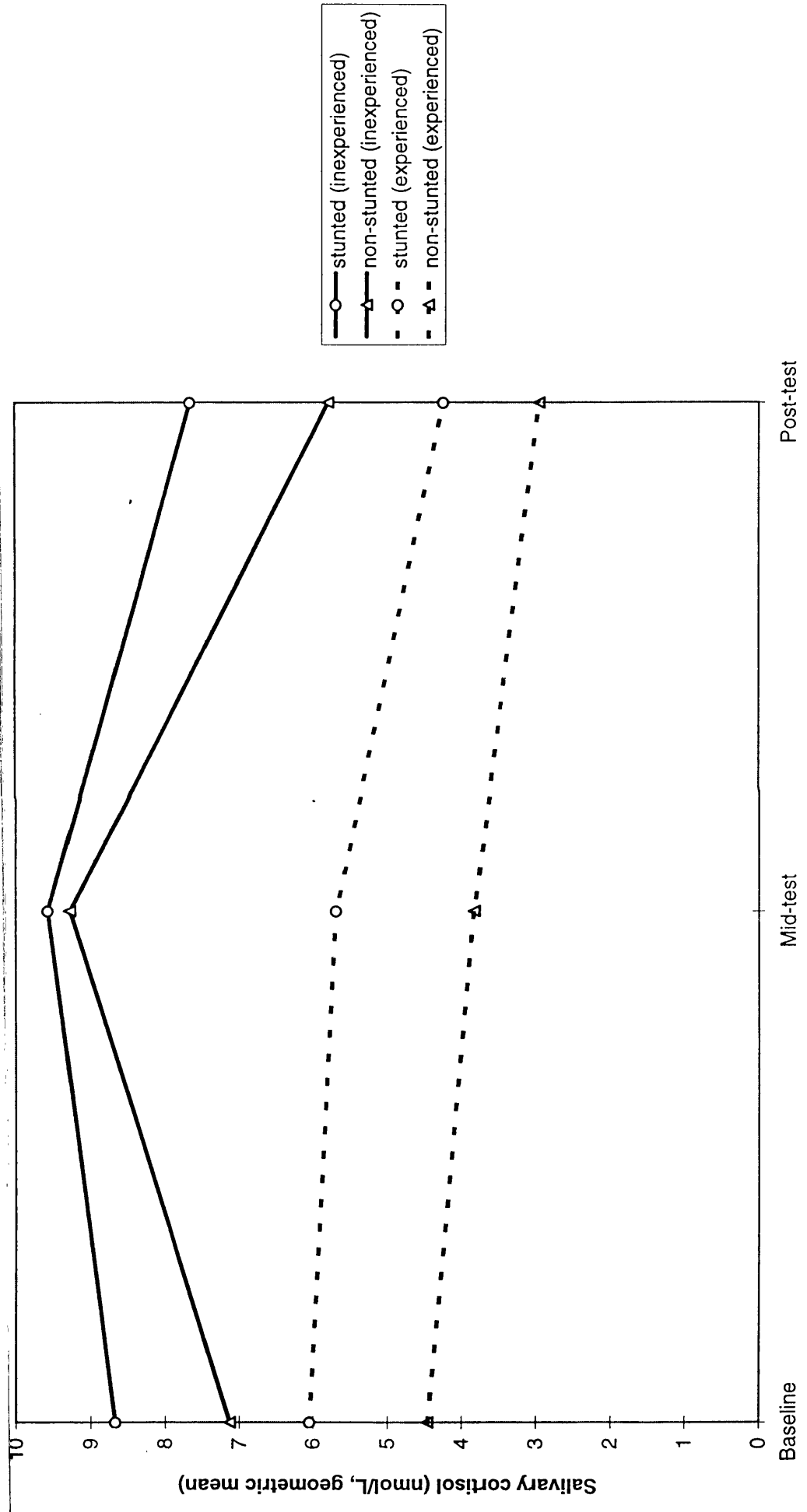
Figure 15: *Geometric mean cortisol levels for stunted and non-stunted Jamaican children from Study 1 (experienced) and Study 2 (inexperienced)*

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It is important to be cautious in claiming an isolated experience effect, because experience is inextricably linked with nutritional status. When comparing non-stunted experienced with non-stunted inexperienced, it is clear that the groups are different not only in their experience, but also in their nutritional status. Specifically, the experienced non-stunted group was significantly taller than the inexperienced non-stunted group (height for age: 0.177 v. 1.022, mean difference: 0.845, $P<0.0001$). Similarly, the experienced stunted group was significantly taller than the inexperienced stunted group (-1.698 v. -2.276, mean difference: 0.578, $P<0.0001$).

With the assumption that there is a linear relationship between height for age and cortisol levels, it is possible to calculate an adjusted "experience effect" with simple calculations. By dividing the B value for stunting effect by the difference in z-scores in the experienced ($0.335 \div 2.72 = 0.123$) and the inexperienced ($0.248 \div 2.45 = 0.101$) groups it is possible to create a mean value for change in cortisol level per height-for-age z-score of 0.112 ($[0.123 + 0.101] / 2$), which means there is a change of 0.112 for every z-score difference in height-for-age. This value then multiplied by the difference in height-for-age between stunted groups ($0.58 \times 0.112 = 0.065$) and the difference between non-stunted groups ($0.85 \times 0.112 = 0.095$) gives a rough value of adjustment for the amount of difference in the B scores for experience effect that could be accounted for by difference in height, resulting in an experience effect of B=0.373 in the stunted group and B=0.475 in the non-stunted group.

Heart rate

The findings for heart rate are very similar to those of cortisol, with stunted children, both experienced and inexperienced, having higher heart rates than non-stunted (**Figure 16**). When the dependent variable was mean heart rate, the "stunting effect" was evident for children from Study 1 (experienced (B=5.17, P=0.021) and children from Study 2 (inexperienced (5.21, P=0.01) analyses, even when controlling for a large number of possible confounders (**Table 26**). None of the additional variables came into either analysis. A substantial "experience effect" is evident in both the stunted (B=4.37, P=0.045) and the non-stunted (B=4.41, P=0.035) groups in response to psychological stress, with a strong combined and experience effect (B=9.6, P<0.0001).

There was also a strong "experience effect" in response to hand grip with children from Study 2 (inexperienced) responding much more significantly than those from Study 1 (experienced) in stunted (B=-6.8, P=0.007) and non-stunted (B=-6.2, P=0.009) children, when controlling for age, sex, BMI, maternal IQ, and stimulation and economic indices.

Table 26: Differences between groups in a multiple regression predicting mean heart rate from psychological testing session from group and several covariates (age, sex, BMI, maternal PPVT, simulation index, economic index)

Comparison group	Variable	Mean heart rate (beats/min)	
		GROUP EFFECT	
		B value (95% CI)	P value
non-stunted, experienced	stunted, experienced	5.169 (0.777, 9.560)	0.021
	non-stunted, inexperienced	4.372 (0.103, 8.642)	0.045
	stunted, inexperienced	9.578 (5.206, 13.951)	<0.0001
non-stunted, inexperienced	stunted, inexperienced	5.206 (1.257, 9.155)	0.01
stunted, experienced	stunted, inexperienced	4.410 (0.306, 8.513)	0.035
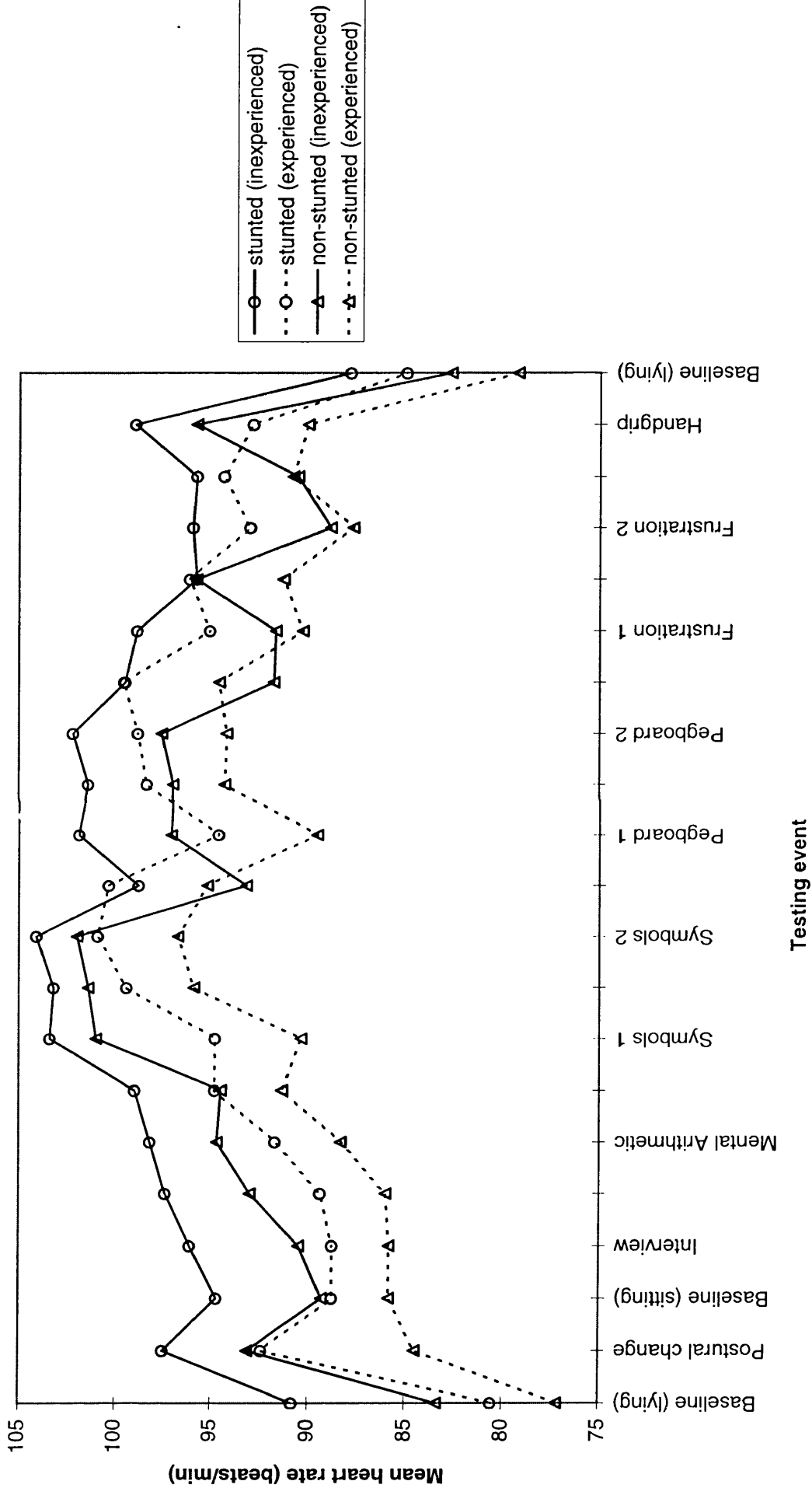
Figure 16: Heart rates for stunted and non-stunted children from Study 1 (experienced) and Study 2 (inexperienced).

Urinary catecholamines

Stunted children, both experienced and inexperienced, had higher levels of cortisol than non-stunted children (**Figure 17**). The "stunting effect" was clear for children from Study 1 (experienced (B=0.30, P=0.018) and children from Study 2 (inexperienced (B=0.25, P=0.049) analyses, even when controlling for a large number of possible confounders (**Table 27**). None of the additional variables came into either analysis. A substantial "experience effect" was evident in both the stunted (B=0.44, P<0.0001) and the non-stunted (B=0.30, P=0.008) groups, with a strong combined stunting and experience effect (P<0.0001).

Table 27: Differences between groups in a linear regression predicting log-transformed mean epinephrine concentration from group and several covariates (age, sex, BMI, maternal PPVT, simulation index, economic index)

Comparison group	Variable	Geometric mean epinephrine concentration (μmol/mmol creatinine)	
		GROUP EFFECT	
		B value (95% CI)	P value
non-stunted, experienced	stunted, experienced	0.305 (0.063, 0.607)	0.018
	non-stunted, inexperienced	0.302 (0.306, 0.835)	0.008
	stunted, inexperienced	0.551 (0.547, 1.090)	<0.0001
non-stunted, inexperienced	stunted, inexperienced	0.248 (0.001, 0.496)	0.049
stunted, experienced	stunted, inexperienced	0.438 (0.226, 0.741)	<0.0001
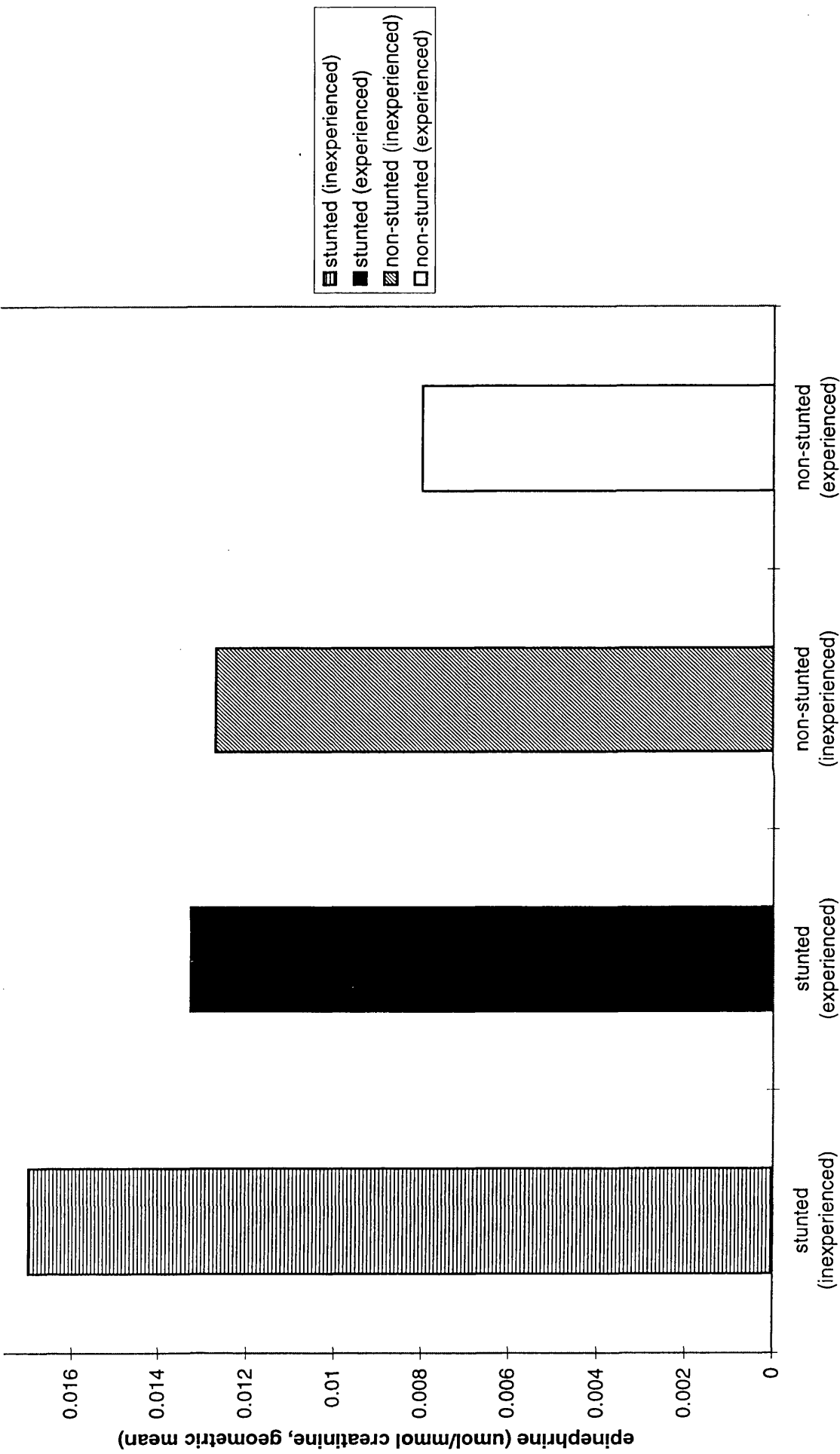
Figure 17: Epinephrine levels (μmol/mmol creatinine) in stunted and non-stunted Jamaican children, from Study 1 (experienced) and Study 2 (inexperienced)

Relationships among variables

There were high correlations across all groups between epinephrine and baseline cortisol (r=0.23, P=0.02), mid-test cortisol (r=0.41, P<0.0001), post-test cortisol (r=0.38, P<0.0001), mean heart rate for psychological testing (r=0.29, P=0.002), postural change (r=0.28, P=0.006) and handgrip (r=0.22, P=0.007).

All cortisol points were significantly correlated with mean heart rate for psychological testing (0.28 < r < 0.37, P<0.0001), and for physiological testing with postural change (0.23 < r < 0.39, P<0.05) and handgrip (0.23 < r < 0.31, P<0.01).

The happy rating was significantly correlated with cortisol level at mid-test (r=-0.21, P=0.024) and post-test (r=-0.21, P=0.029), and with epinephrine (r=-0.38, P<0.0001) and dopamine (r=-0.21, P=0.026). The inhibition rating was significantly correlated with epinephrine levels (-0.337, P<0.0001), as was responsive vocalization (r=-0.22, P=0.01).

Chapter 8: Results from interviews about problem situations, punishment, school and social roles with Jamaican children

Introduction

The interview consisted of 12 open-ended questions about school, social roles and problem situations, designed so that the children could freely discuss whatever issue first came to mind pertaining to each particular topic. The questions were organised so.that the ones about problem situations came last, in order to avoid biasing the children towards answering the first six questions about school and social roles with answers relating to punishment, violence, or aggression.

Although the responses were coded into several different categories, the analyses reported in this thesis focus on the children's perceptions and reports of aggression and violence, because that topic pertains most directly to stress level. The answers relating to violence and aggression were examined closely because they are dramatically informative about the psycho-social stressors that exist in the lives of children living in downtown Kingston.

The results are organised by question, beginning with what happens at school, followed by perceptions of social roles, and then interpretations of problem situations. Words from the Jamaican patois are defined where necessary in parentheses immediately following the word. Listed in parentheses after each quotation is each child's nutritional classification and sex. These identifiers were included to illustrate the range of responses across nutritional group and sex in terms of their expression of violence and aggression.

Interview responses

Of the 123 children interviewed, 112 (91%) mentioned something relating directly to violence or aggression in response to at least one of the twelve interview questions.

School, teachers and principals

In response to the first question in the interview ("What happens at school?") almost half of the children (n=56) reported something relating to violence, aggression, punishment, beating, shooting or killing (**Figure 18**).
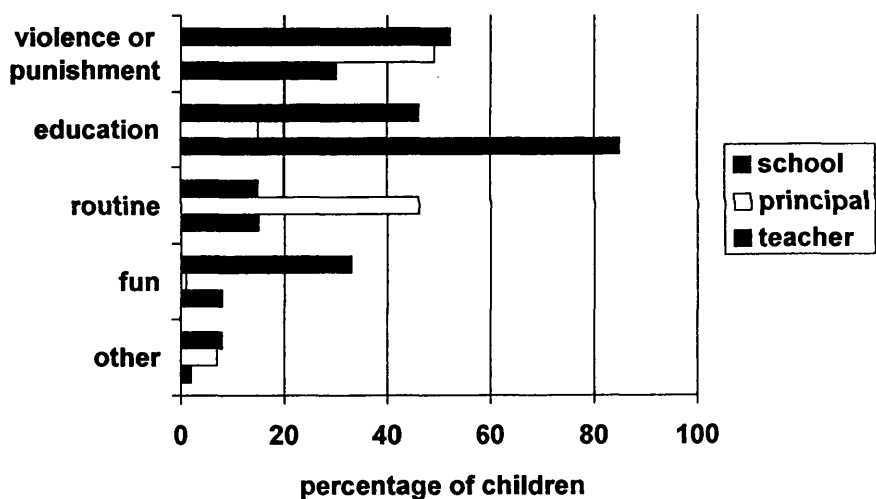
Figure 18: Percentage of children responding in each coding category in response to questions about "What happens at school?", and "What does a teacher/principal do?"

The majority of responses about violence related to fighting with friends.

Some of the more descriptive responses of this type were:

"The children fight. They kick up one another. They climb the fence and tief [steal] people junk food. They stab up one another [with a pencil].... They cuss bad words. Them don't obey dem [their] teacher.... And they make up lies in the class and them spit in the children's face, and some of them read them book and some of them write slow and some of them write fast.... And them always do bad things. And them always telling untruths on other people and them always running and slapping in the class." (female, stunted)

"Me and mi [my] friends dem [they] fight." (male, stunted)

"We fight. And stab people. And stole money. And tief [steal] people pencil." (male, non-stunted)

"They fight, miss. They romp. They shot in they one another eye. They kick. They romp. They box. Stab one another with pencil. They thump in a eye." (female, stunted)

Bullying and victimization were frequently mentioned. In most cases, the

bullying was described as something happening to other children.

"Sometimes the big children kick the small children. And they fight. And throw away the lunch. And kick." (male, non-stunted)

"Miss, dem [they] beat up pickney [children], dem beat up likkle [little] pickney." (female, non-stunted)

The other descriptions of bullying revolved around the descriptions of experiences of

victims. There were no cases in which children admitted to being bullies, or

described experiences in which they bullied other children.

"Sometimes them go into my bag and tief my lunch money. And when I went to the toilet and I left my book, them take it up and tear it. And when I have my pencil them say it's theirs and start to fight me." (female, non-stunted)

"One time when I sit down, the pickney [children] them come trouble me. And then we do our work, them come trouble and stab me with them [their] pencil." (male, non-stunted)

"Sometimes my friend beat me up." (female, stunted)

"Stab into my head. Kick me. Throw me. Kick me. Shoot me down. Kick up mi [my] head. Beat me. Cut pon [upon] my hand. Them throw me on mi [my] face. Shove me pon [down onto] the ground." (male, stunted)

"Sometimes some children who don't bring their lunch ask you for your lunch, and if you don't give it, them beat you up." (female, non-stunted)

Several children described fighting along with playing when describing what happens at school, which suggests that the fighting may be considered a type of play. For example:

"People can fight. And play and kick. And steal and run up and down and give a whole heap of trouble." (female, stunted)

"Children's fight, miss. Stab and play." (male, non-stunted)

"War. Children fight and stab them one another. Gunshot fire. Play games. Share our food. Play ring games." (female, stunted)

"The children dem fight. And stab. And thump one another. And them play." (male, stunted)

"Children fight at school. Play. Line up." (female, non-stunted)

"...And we play at the yard. We play games--sometimes we fight. And we used to play dandy shandy." (male, non-stunted)

Many of the responses that mention violence in the context of other things that happen at school.

"Them fight. They cuss bad words. Some of them play. Try to read. And do them [their] work." (male, stunted)

"They do work. Sometimes them fight. And sometimes they stab people." (female, non-stunted)

"Fight, play, cricket, football, kicking." (male, non-stunted)

"Sometime I don't do my work so good. But sometime school is good. But the children will fight with me. And I have to hit them back. I do my work. And then I look for my pencil if I lose it." (male, non-stunted)

"You do your work. Eat your lunch. Take private lesson. Do homework. Play. Teacher beat you. Do maths. English. Mathematics. Social study." (male, stunted)

A small percentage of children referred to community violence when asked about what happens at school.

> "War. Children fight and stab them one another. Gunshot fire. Play games. Share our food. Play ring games." (female, stunted)

> "They come and fire, and soldiers search up the school. And them shoot a man right at our gate." (male, stunted)

> "Them fire shot. The bad man them run up and down and fire shot and kill people. And the police them run after them and fire shot after them. And sometimes bad man come and fire shot after them." (female, non-stunted)

> "Them come a school and sell guns." (male, stunted)

The familiarity of these children with community violence and aggression is not surprising considering that all the schools that children in the study attended were in downtown Kingston, and did not always have secure boundaries separating the schools from the community.

There were no differences between nutritional groups in terms of experience with violence or aggression.

Perceptions of social roles: teachers and principals

The most commonly reported activities for teachers revolved around teaching (**Figure 18**), with teachers functioning as educators of both school subjects and good behaviour.

> "She write on the blackboard. She give me work. She give we studies. And reading. Sometimes she give we phonics." (female, non-stunted)

> "She give we work on the blackboard. She make we sit and behave ourselves. She set work at the blackboard. She say we must behave ourself and sit down. And she register our name." (male, stunted)

Principals were also implicated in the education process, although much more minimally than teachers, and more likely in a supervisory context.

"I don't know, miss. Sometimes he will walk around the class, look at what us, the children, are doing. Then he will leave, go for something, come back, and then he go in his office, go to the staff room and look at the papers. (female, non-stunted)

Thirty percent of the children mentioned something about violence or punishment when describing what a teacher does, though usually in the context of other activities. For example,

"She learn us. And tell us that we must not give any trouble. And we must behave ourselves. And sometimes she lick [hit] some of us when we don't listen to what she is saying. And when she talk some of them walk up and down in the class. And make noise when Miss is teaching." (male, non-stunted)

"She writes something on the blackboard to give we. And she mark the book. Sometimes she help sweep up the classroom. Sometimes she beat me. Sometimes she read." (female, non-stunted)

"She teach the class. She keep the class quiet. She beat. She talk to the next teacher. She give we class work." (male, stunted)

"She teach. And she lick [hit] the children. And sometimes, when the children don't do any work, she throw them out of the class." (female, stunted)

The principal was much more often described as punishing and exhibiting violent behaviour than the teacher, as evidenced by the fact that almost half of children described this role.

"Beats us with a cane." (female, stunted)

"Like when the children throw the bag on the floor she beat them. And tell them to take it up. If they give trouble, they get sent to the principal." (male, non-stunted)

"Some of the time, he beat the children, dem. And he come up a class and tell the children to sit down in them seat." (male, stunted)

"Beat children when they do rude things. Tell the teacher what to do." (female, non-stunted)

As is clear from **Figure 19**, most children who were interviewed perceive their parents to be doing housework and work outside the home. Seventy-six percent of children reported that their mothers were tending to the house and children, and 36% reported the same duties for their fathers. Similarly, 36% reported that their mothers worked outside the home and 64% reported those roles for their fathers.
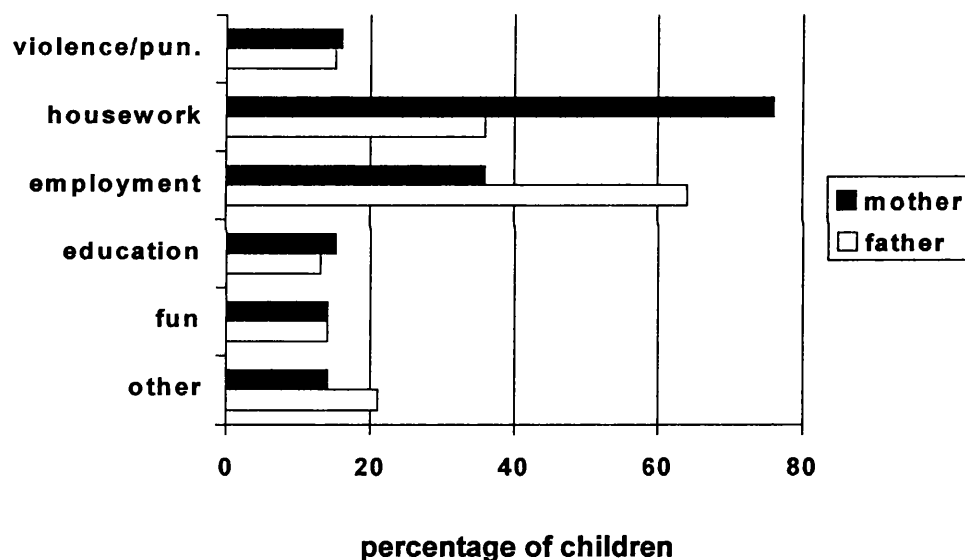
Figure 19: Percentage of children responding in each coding category in response to questions about "What does a mother/father do?"

Mothers and fathers were equally as likely to be reported to be violent or to punish, and the punishment and violence was usually reported in the context of a variety of activities, but not always.

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[Mother] "Beat, cook, wash, work." (male, stunted)

[Father] "Beat. Him take the rubbish out the back yard." (female, non-stunted)

[Father] "Lick [Hit] you. Thump [Hit] you down. Beat you on your bottom." (female, stunted)

[Father] "Look after them children. Some of the father beat you sometime. And beat your mother too. Sometimes they take drugs. Sometimes they kill people. Touch up them children when they drink rum." (female, stunted)

The responses listed above describe beating as a characteristic parental behaviour, unrelated to child behaviour. In many cases, however, children seemed to understand the relationship between their behaviour and the ensuing punishment.

"Mother look after them children. Buy clothes and give them. Buy shoes. Pay school fee. Buy uniform cloth. Buy dress to you wear go to church. Buy shoes to you wear go to school. Buy slippers for you wear. Sometimes when you don't obey, they will beat you." (female, stunted)

"She work. She look after good. Sometimes she beat we when we do wrong things. Sometimes she make me run round the yard." (male, stunted)

"Him work. Him bring food for we. Sometimes when we give trouble him beat we." (male, non-stunted)

"Bathe her children. Comb their hair. She look after them. Wash the dishes and tidy the house. Wash their clothes. And if you tear your clothes, she will patch them up. When you [are] rude you get spanking." (female, non-stunted)

"My father go to work. He have a good job. Anything I do wrong, he spank me. And sometimes he go to work, and give me lunch money." (male, non-stunted)

In this sample of children, parents seemed to have a role not just in administering punishment, but also in educating their children about violence and how behave in response to aggression.

"A father come to the school and say nobody [should] lick [hit] me on my belly cause my belly hurt. And them kick me around. Father say fi [to] lick them back." (male, stunted)

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[Mother] "Care us. Look after us. Teach us manners. Beat us. Send us out. Teach us manners. And cook our dinner. Share out dinner. Teach us prayers. Talk to us. Tell me, 'Do not fight.' 'Do not use your foot on girls.' 'Do not kick girls.' 'Do not kill.' 'You must love.'" (male, stunted)

"A father care them child. And tell them that they must do them school work. And must not do anything wrong -- like fighting in the classroom. And also not to run up and down when teacher is talking. And chatting." (male, non-stunted)

In responding to the questions about the roles of mother and father, there was

a slight tendency for boys to report more violence and aggression that girls.

Perceptions of social roles: policeman

The majority of responses (54%) to the question, "What does a policeman

do?" included a mention of violence and punishment (**Figure 20**).
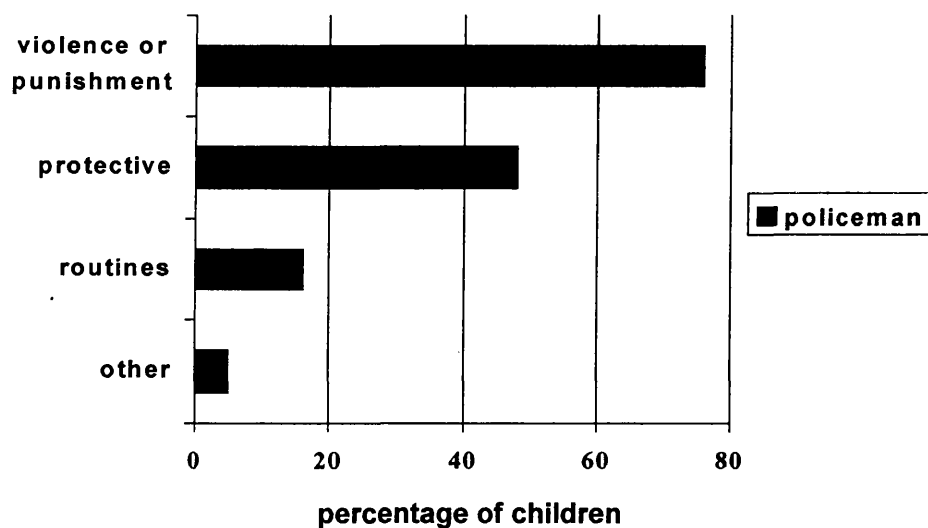
Figure 20: Percentage of children responding in each coding category in response to question about "What does a policeman do?"

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Some typical responses included:

> "The policeman always fire shot and shot the people. And they always kill the people." (female, stunted)

> "Catch the criminal. And nail them to the wall. Sentence you to jail." (male, non-stunted)

> "Beat people with them baton. Shoot dem. Tell them go to jail." (female, stunted)

> "Shoot people." (male, non-stunted)

> "Fire guns. Kill peoples. Lock them up." (female, stunted)

> "Lock up people. Beat people. Kill people. Talk to people. And love people." (male, stunted)

> "He help people to cross the road. And stop people from do the bad things. And locks up people. And shoot people." (male non-stunted)

A small number of children appeared to have been affected directly by police violence.

> "Shoot my father, brother. Beat me. Kick me. Beat me up." (male, stunted)

> "He lock up people. And him box my Christian uncle." (male, non-stunted)

> "Two men was fighting in my yard. Shot one another and the police came and kill." (male, stunted)

Problem situations

Of the 123 children interviewed, 103 (84%), children reported some sort of physical punishment for one of the six hypothetical problem situations. Of those children responding with reports of corporal punishment for any of the six, 77 of 103 (75%) said they would get beaten when they were asked, "What happens if you get into trouble at home?" The next most likely situation to inspire physical punishment, either from a parent or teacher, was tearing the uniform, followed by being late for school, losing a school book, having money stolen, and losing lunch money.

<u>Punishment at home</u>

In response to asking what happens if they get into trouble at home, most children answered simply that their mothers will beat them. Other punishments were also reported.

"My mother will beat me and send me to my bed." (female, stunted)

"My mother beat we or punish we." (male, stunted)

"Mother will beat you and cuss you." (female, non-stunted)

"My mother cuss me and lick [hit] me with her hand." (male, non-stunted)

"My mother beat me. And send me to mi [my] bed." (female, non-stunted)

"My mother will beat me. And me have to run. I have to hide." (male, stunted)

In one extreme example, a child reported,

"My mother beat me. And kick me. And fling stone after me. Turn me upside down and beat me. And run me down with stone. And lick me up with stick. She cook and don't give me any." (male, stunted)

As shown in the above examples, in most cases of punishment at home, the mother was the one beating. However, other family members were also likely to get involved in some cases.

"My father will beat me. And tell me fi keep off the road. And tell me fi stay in the house. And tell me to go and read my book." (male, non-stunted)

"Father beat me. And him tell my grandma, and my granny beat me." (female, non-stunted)

"Your cousin beat you or your uncle or your brother." (female, stunted)

"If it's my brother he beat me up. And if it's my sister, she beat me. And if it's my mother, she beat me." (male, non-stunted)

"Mom beat me. My cousin will tell my father, and him beat me." (female, stunted)

"My grandmother beat me." (female, stunted)

"My brother beat me." (male, stunted)

In only a few cases did the beating seem to be related to parental teaching because in most cases, the children just reported being beaten without discussing the implications. However, sometimes there were clear lessons that were learned.

> "If I get in trouble at home my mother will beat me. And my mother say I must not give no trouble. And she say when I grow up I must not give trouble. And be a decent boy." (male, non-stunted)

> "Sometimes I get into trouble, she beat me, or she make me stay in my room and don't come out back. And sometimes she say, 'You're nasty, and I love you too much for you to get yourself in that kind of trouble.'" (female, stunted)

> "When I get in trouble, my mummy will ask me why I do it. And she will beat me. And sometimes she give me a second chance. And she will tell me that it's not nice. And that I should't do it. Sometimes she will beat me though." (male, non-stunted)

> "Your mother will beat you. And you wouldn't want to do it again cause it's not a nice thing to give any trouble at home." (male, stunted)

Punishment at school

When children were asked about what would happen if they get into trouble at school because they were late or otherwise causing trouble, the teacher and/or principle was likely to punish by beating. Children described being put in a dark room and beaten, having to stand out in the sun with their hands atop their heads, just being beaten in front of their peers.

> "Sometimes she beat us, and sometimes she give us books, heavy books to put on your head for punish you." (female, stunted)

The most mild punishments involved having to pick up rubbish in the school yard, or being reprimanded in front of the class. In most cases, the punishment did not appear in any way to be related to the infraction, and beating was by far the most common punishment for every offense.

In some cases, the punishment for getting into a fight is to be beaten, which illuminates the circular nature of violence.

> "Your mother will come and ask you what is the problem. And you will tell her you was fighting. And you will get a hit. And your nose would start bleeding. She would buy a Band-Aid and put it on my nose. And tell me if I fight again, she will beat me again." (male, non-stunted)

Similarly, a girl reports her mother's words of advice on what to do next time,

> "Sometimes she punish me and tell me I must not trouble them. And if they trouble us, I must trouble them back." (female, non-stunted)

Children seem to be learning that violence is an appropriate way to deal with problems, from their teachers, parents, and from their peers.

> "Sometimes when I'm outside the big boys in the bigger grades come and beat us up. And we tell their teachers. And the teachers beat them."

> "The children want to fight me and I fight them back. And when we go inside the children tell on me and the teacher beat me, and I tell them I'm going to go outside and beat them up cause they tell on me." (female, stunted)

> "Anytime we fight or stab anybody she come and beat we." (male, stunted)

> "When mi go a school, them take away my money. Tell my big bredder fi beat them up. My mother beat them. Mi brother lick me back, and I lick him back and he kick me and him hit me in the back. My mother send me in the house. Then she beat my big sister." (male, stunted)

Physiological correlates of reporting aggression

There were no significant correlations between the violence rating and any of the physiological variables (epinephrine, norepinephrine, dopamine, mean cortisol, mean heart rate) and any socio-economic (mother IQ, subject IQ, birthweight, possession rating, economic rating), anthropometric (height for age, BMI), sex or age with all groups together. There were also no differences between groups in the quantity of violence reported. The study was not designed to investigate these questions or hypotheses, however.

Chapter 9: Discussion of Jamaican studies

The discussion of Study 1 and Study 2 will begin with an overview of the main findings in the following categories: socio-economic characteristics, behavioural measures, salivary cortisol, heart rate, urinary catecholamines, and perceptions of violence and aggression. The internal and external validity of the studies will then be addressed, followed by a discussion of potential physiological and psychological mechanisms. Then the cognitive, immunological, psycho-social and physical implications of the findings will be presented.

Overview of findings

Introduction

Based on two separate studies, stunted Jamaican school-children had higher salivary cortisol levels, heart rates, and urinary epinephrine concentrations during a test session of psychological and physiological stressors than did non-stunted children who were the same age and lived in the same poor neighborhoods of Kingston, Jamaica. These physiological results support the hypothesis that the hypothalamic-pituitary-adrenal axis and autonomic nervous systems in stunted children may have been altered during development. The stunted children also vocalized less, and were more inhibited, less happy, less attentive and more frustrated than the non-stunted children. All children, stunted and non-stunted, reported a substantial amount of experience with violence, aggression and corporal punishment from peers, parents, teachers and principals.

Socio-economic characteristics

Children in both studies were matched for neighborhood, which was very important given the complex relationship between nutrition and the socio-cultural environment (Grantham-McGregor, 1984). To control further for environmental factors, extensive measurements were obtained of potentially important characteristics, such as parental education and occupation, housing and water status, and stimulation, all of which were included in statistical analyses of the data. These covariates were included even though not one was significantly related to any of the outcome measures. Thus, the reported analyses were very conservative.

There is evidence that the environment is associated with physiological stress levels in children (Flinn and England, 1997) and animals (De Jonge et al. 1996), suggesting that environmental factors could contribute to differences between the groups reported in these studies. However, most group differences, including salivary cortisol level, heart rate, urinary epinephrine, and inhibition rating remained significant after the inclusion of covariates. The interactions between behaviours and physiological status that emerged in Study 1 also remained significant after inclusion of several covariates (socio-economic indices, maternal and child IQ).

Children in Study 1 were matched for neighborhood whereas children in Study 2 were matched for school. The data show that the stunted and non-stunted children from Study 1 differed significantly in terms of socio-economic markers while those in Study 2 did not as much, suggesting that school-matching may be a better technique for ensuring similarity of background. It could also be that matching that occurred at two years old was not as valid eight years later. The fact that the stunted and non-stunted groups in Study 2 did not differ significantly suggests not only that the experience effect may have overwhelmed the nutrition

effect, but also that poor social background may contribute to the significance of the physiological differences in the first study. However, the analysis with the four groups together clearly indicate that the nutrition effect is present in both studies, regardless of experience, and controlling extensively for social background, current nutritional status and maternal stimulation.

Behavioural measures

For the first time, a wide range of behaviours of stunted school-children has been observed, described and replicated in two studies with Jamaican children. Stunted children talked less, were more inhibited, less happy and less attentive than non-stunted children, and these differences remained significant with the inclusion of socio-economic variables. Stunted children also tended to use fewer strategies and be less persistent when faced with a frustrating task. There was no effect of novelty or experience on any of the behaviours.

In Study 1, the relationship between behaviour and cortisol levels differed according to group. Stunted children with higher cortisol levels vocalized less and were more inhibited than those with lower levels of cortisol. The opposite relationship existed in non-stunted children with higher cortisol levels, who had high levels of vocalization and low levels of inhibition. During heightened physiological arousal, non-stunted children adapted better to the situation by talking more and relating comfortably with the interviewer, rather than being withdrawn and silent, a relationship that is consistent with previous research with normal children in developed countries (Gunnar and Nelson, 1994). These findings were not replicated in Study 2, most likely because of the large novelty effect associated with cortisol.

Salivary cortisol

Stunted children had significantly higher levels of salivary cortisol than non-stunted children, and this finding was replicated in both studies, controlling for social background. The salivary cortisol concentrations reported in this study are similar to those found in children of the same age from Dominica (Flinn and England, 1997), Ethiopia (Dobrowolska and Panter-Brick, 1998), and Nepal (Panter-Brick et al. 1996b), and from the developed world (Gunnar, 1993).

Given the data on salivary cortisol responsivity to psychological stressors (Kirschbaum and Hellhammer, 1994), it is surprising that the Jamaican children did not appear to have an adreno-cortical reaction to the testing experience. The only group exhibiting a tendency to react was the non-stunted group from Study 2 (inexperienced), although the findings were not significant. Very likely, the children in both studies had already begun to respond to the "stress" of being collected at school in a University vehicle, being transported to the University, and then waiting for the test to begin. Due to this constraint in study design, it is impossible to say if any of the groups reacted adreno-cortically to the testing situation.

A very substantial experience effect was evident, with the children from the longitudinal study (Study 1) having significantly lower cortisol levels than the newly recruited children. This experience effect was the same size regardless of nutritional status (stunted or non-stunted). Interestingly, the B value for the combined effect of the stunting and experience effects appeared to be a summation of each individual effect.

Heart rate

Stunted children had higher heart rates than non-stunted children, even when controlling for a substantial number of covariates. There was also a substantial novelty effect that was demonstrated by comparing groups of the same nutritional status who had different experiences. All four groups responded cardiovascularly to the psychological testing, which was to be expected, given the literature about the effects of mental arithmetic and other testing on cardiac functioning (Cacioppo et al. 1995). There is no evidence that the stunted and non-stunted differed in their responsivity to psychological stressors.

The stunted group in Study 1 had heightened reactivity to physical stressor (postural change), even when controlling for a number of covariates. This finding was not replicated in the newly recruited group.

Urinary catecholamines

Stunted children had significantly higher levels of urinary epinephrine than non-stunted children, when controlling for a wide variety of socio-economic factors. The inexperienced stunted group from Study 2 had higher levels of norepinephrine than the inexperienced non-stunted controls. This difference may have been due to the overall heightened level of reactivity in the newly recruited group. There were no differences between the stunted and non-stunted groups for urinary dopamine excretion.

As expected, there were high correlations between epinephrine and cortisol levels (Long et al. 1993), which supports the internal validity of the measurements.

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Effect of novelty

The effect of novelty was clearly evident within a homogenous population,

when comparing the longitudinal (Study 1) with the newly recruited (Study 2)

Jamaican children. The first-time Jamaican subjects had higher cortisol levels, heart

rates and epinephrine levels than their experienced peers, suggesting that there is a

blunting of the novelty effect that occurs in response to repeated stimulation.

Habituation to a stressor (being handled at a young age), has been shown to occur in

both animals (Meaney et al. 1988) and humans (Gunnar et al. 1992).

Perceptions of violence and aggression

Interviews revealed a very substantial amount of violence and aggression in

the lives of Jamaican children. Given the socio-economic differences between

stunted and non-stunted children, it was surprising to find no differences between the

groups in terms of their reporting of violence. Low socio-economic status has

consistently been associated with childhood violence (Dodge et al. 1994; Kolvin et al.

1988; Farrington, 1978), and has been associated with harsh parental discipline, lack of

maternal warmth, exposure to aggressive adult models, and reduced cognitive

stimulation (Dodge et al. 1994), suggesting that aggressive behaviour may be a

response to the stressful environment associated with low socio-economic status

(Kupersmidt et al. 1995). Having a large family or number of children may also affect

a child's development by depreciating the quality of family interaction.

Children with difficult temperaments have increased behavioural problems

(Tschann et al. 1996) and high levels of aggression (Caspi and Silva, 1995; Sanson

et al. 1993; Kingston and Prior, 1995). However, the effect of a child's temperament

has been shown to be mediated by several factors. For example, higher levels of

aggression have been observed in children with temperamental difficulties when the

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mother endorses an aggressive parenting style (Dukewich et al. 1996), when there is high level of conflict in the home (Tschann et al. 1996), and when the family has a low socio-economic status (Sanson et al. 1993).

Given the Jamaican cultural context, it may be dificult to interpret levels of violence and aggression described by children. However, these data do clearly show the large amount of violence that children are facing in many aspects of their lives.

Validity and limitations of findings

Before interpreting the implications of the data, or discussing their implications it is important to consider the external and internal validity of the findings.

Validity

The validity of a measure or finding reflects the extent to which that measure or finding reflects what it is supposed to measure (Hennekens and Buring, 1987; Abramson, 1994; Bowling, 1997). Two important types of validity must be satisfied before findings can be considered valid either for the children tested, or for the target population: internal (includes statistical-conclusion validity and construct validity) and external validity (Cook and Campbell, 1979).

Internal validity

Statistical-conclusion validity

The statistical-conclusion validity determines the confidence with which an investigator may claim that a relationship exists between the independent and dependent variables. Threats to this type of validity include: low statistical power, violations of assumptions of statistical tests, use of multiple statistical tests, low reliability of the dependent and independent variables, random heterogeneity of the subjects, and confounding (Cook and Campbell, 1979).

A study has statistical power if it identifies differences that actually exist as statistically significant. Low statistical power usually results from small sample sizes, and increases the risk of type II errors (false negatives). Also, when a study has low power, the analyses are often able to withstand only a limited number of covariates. For this reason, it may not be possible to use multiple covariates, even though this may be the most statistically valid analysis. Study 1 drew from a longitudinal cohort, which meant that the sample size was limited to the children who had been successfully followed for seven years.

Unfortunately, the attempt to expand the sample size with newly recruited children (Study 2) was unsuccessful because of the overwhelming effect of novelty. There was a clear "experience" effect, causing the children who had never been up to the University to have substantially heightened physiological measures. Therefore, the four groups (stunted experienced, non-stunted experienced, stunted inexperienced and non-stunted inexperienced) had to be kept separate. However, it was possible to test the differences among the four groups statistically with a dummy variable design.

Clearly, both samples were selected based on quantitative methodological techniques. Even so, the responses to the interview yielded very interesting results. In order to capture the true depth of experience of urban Jamaican children with aggression, however, information-rich, purposeful sampling would have been much more appropriate sampling strategy (Patton, 1990).

The data reported here were analyzed using repeated measures ANOVA and ANCOVA whenever possible, because repeated measures analyses test hypotheses about the relationships between dependent variables measured multiple times and the conditions under which they are measured. The assumptions of homogeneity of

variance, normal distribution and parallel slopes for the covariant analyses were also satisfied. Similar assumptions were satisfied when linear regression was used.

During the course of analysis, many statistical tests were undertaken, potentially increasing the number of type I errors, or false positive findings. To reduce this likelihood, repeated measures analyses or analyses using mean values were performed as often as possible, and results were not considered significant unless it was possible that they were likely to occur more often than chance.

If the independent or dependent variables are not reliable, then variability may increase, which decreases the chance of finding true significant differences. In all studies reported here, the selection criteria for subjects were a series of standard, objective measures. The tests were administered in a standard format; and the same two testers carried out all the tests. Reliabilities were established during piloting and were consistently high. The test session was highly standardized, although the timing of saliva collection was not always consistent. The testing record sheet for all studies included a checklist to ensure that every step of the protocol was completed (**Appendix II**).

The testing environment in Jamaica was highly standardized because children were brought from school to the University, and were always tested in the same room, and were measured for baseline levels in a quiet part of the hospital ward. The only difference in testing experience among subjects was that some were tested immediately upon arrival and others had to wait for approximately an hour before testing. However, equal numbers from both groups were tested first and second, which minimized the effects of order.

In order to reduce error variance and the random heterogeneity of the subjects, it is important that study subjects be selected from homogenous

populations. In the studies reported here, children were carefully matched for age, sex, neighborhood (Study 1) and school (Study 2). Due to matching for neighborhood and/or school, the study samples were homogenous in terms of age and socio-economic status.

Construct validity

Construct validity refers to the extent to which theoretical inferences and interpretations can be made from empirical findings, and a study with construct validity has well-defined indepedent and dependent variables (Barrett, 1984). The threats to construct validity include pre-operational explication of the constructs, mono-operational and mono-method biases, and experimenter expectancies (Cook and Campbell, 1979).

Pre-operational explication of the constructs refers to the fact that constructs must be fully conceptualized before the onset of the study, so that there can be clear conclusions about the relationships between the dependent and independent variables. In the Jamaican studies, this problem was avoided by using extensive piloting, during which all measures were tested and crystallized. Thus, during testing, there was no confusion about what the behavioural constructs were. The physiological measures were carefully selected to reflect different parts of the same phenomenon, stress reactivity. The intercorrelations among the physiological variables attests to these relationships.

Piloting addressed the problem of inadequte conceptualization of the variables, which refers to incomplete definitions of the variables. Since all behavioural measures were shown to be normally distributed, the construct ratings were clearly reflecting behaviours across the population.

Mono-operational and mono-method biases, resulting from under-representation of a construct, was prevented by using different methods of measuring responsivity. Specifically, a host of related behaviours were rated, and three related physiological outcomes were measured, which gave multiple perspectives on the same phenomena. Bivariate correlations showed that the behaviours were often related to each other in the directions hypothesized. Correlation among some physiological and behavioural variables also confirmed the lack of mono-method biases.

Since the testers were blind to the child's nutritional group, experimenter expectancies were not considered to be a problem. The large age range of the children involved in the study masked any obvious height differences among children. Sessions were videotaped, which allowed for post-hoc confirmation of validity.

External validity

External validity refers to the extent to which findings can be generalized to the target population (e.g. Jamaican children), and to different populations from different settings (e.g. stunted children from another developing country).

The findings from Study 2 clearly replicate the findings from Study 1, establishing the external validity of the findings within the stunted and non-stunted populations of downtown Kingston. Whether these findings are representative of Jamaica as a whole is difficult to assess, given the differences among children from rural and urban contexts.

The extropolation of these findings from urban, Jamaican school children to children of the same age and nutritional status in other developing countries is not

possible. There are a multitude of factors relating to nutrition in a particular country, given the well-established interactions between nutrional and environmental factors.

The setting under which the study was conducted was designed to be ecologically valid since Jamaican school children are orally examined at school. However, the ecological validity was limited since the studies were conducted under standardized testing conditions by unfamiliar testers. As is clear from the results, the experience of coming up to the University for testing had a tangible impact on the children who were unfamiliar with the procedure.

In summary, this study had robust external validity in the Jamaican context. It is impossible to say which, if any, of the findings can be generalized to other populations of stunted children in developing countries.

Limitations

The interview session was designed to be a standardized, open-ended, inductive analysis, because it involved exploring genuinely open questions rather than testing any theoretically driven hypotheses. The strengths of this type of interview are that the responses are highly comparable, the data are complete for every person, the interviewer bias is reduced, and the organization and analysis of the data is greatly facilitated (Oppenheim, 1992; Patton, 1990). However, using this technique offers little flexibility, and the standardized wording of questions may constrain or limit the natural application of responses. This study suffered particularly from the constraint of interview setting, because the setting itself was originally designed to be a psychological stressor.

Potential mechanisms

The findings strengthen the interpretation that poor childhood nutrition is a factor directly related to heightened physiological activation.

Socio-economic environment

However, it is possible that there were other unmeasured socio-cultural differences that may have accounted for some of the differences. Work with children in Dominica has shown that children living with both parents, a single parent (with kin), or grandparents have lower levels of cortisol than children living with stepfathers, a single parent (without kin), or distant relatives (Flinn and England, 1997). These questions were not explored in this thesis due to lack of data.

Researchers have shown differences in anthropometry and neuromuscular reaction time between children who are short and from areas of high SES, suggesting genetic shortness, and children who are the same height for age from areas of low SES, who are more likely to be stunted and short for environmental reasons (Henneberg et al. 1998). The stunted children had shorter limbs, narrower shoulders, thinner skinfolds and had poorer neuromuscular reaction time than the genetically short children. These findings suggest that there are several socio-economic factors which could relate to the anthropometric and physiological correlates of growth retardation, which could then independently contribute to outcome.

Birthweight

Size at birth has been found to have a quadratic relationship with hypothalamo-pituitary-adrenal function (Clark et al. 1996), with low and high birthweight babies having high cortisol levels in later childhood. In the present studies, there was no significant linear or quadratic relationship between size at birth and cortisol levels, and the stunted children's cortisol levels remained higher than the non-stunted group even with control for birthweight. The findings therefore

suggest that factors associated with post-natal growth retardation may alter adreno-cortical activity independently of birthweight.

Growth retardation

Growth retardation is multifactorial and may be explained by several different factors including poor nutrition, high levels of infection, and problematic mother-infant interaction (Waterlow, 1994b). Although no specific nutrient has clearly and consistently been linked with linear growth retardation (Allen, 1994), poor nutrition alone can cause stunting, particularly in developing countries (Lampl and Johnston, 1978). Nutritional deficiencies were at least partly responsible for stunting in the children in Study 1 because a sub-sample of the larger group of stunted children in the original study responded to nutritional supplementation in early childhood with linear growth improvements (Walker et al. 1991). Since the stunted children in Study 1 had failed to catch-up in growth since early childhood, it is not clear whether the altered stress response system in stunted children was solely a consequence of adverse early childhood conditions, or if continuing poor conditions may also be a contributing factor.

Given the research on the development of stunting, it is very likely that the children in Study 2 who were stunted at age nine had also been stunted in early childhood. Thus, the same difficulty exists in evaluating the mechanism. Previous work has shown that conduction velocities in peripheral motor nerves, which are adversely affected by undernutrition, finish developing by 3 years old, suggesting that responsivity may be set in place from a very young age.

Hormone production and activity

The physiological differences that were measured in stunted children could be due to increased production or decreased metabolism of neuro-transmitters or

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hormones, or to altered receptor sensitivity. Specifically for cortisol, there may have been differences in CRH production, levels of CBG, ACTH response, the affinity or density of receptors or the efficiency of the negative feedback regulation (Fuchs and Flugge, 1995; De Kloet, 1991; Meaney et al. 1988). Some evidence from animal work suggests that chronic stress (i.e. unpredictable physical stressors consistently for three weeks) reduces the number of glucocorticoid receptors in the brain, without changing receptor affinity (Sapolsky et al. 1984).

These data also illustrated a "floor effect" with a limit to the extent of down-regulation, despite extending length of exposure to stressor or directly injecting corticosterone. Receptor levels return to normal within a week of treatment cessation, suggesting that the down-regulation resulting from chronic stress may be due to reductions in receptors per neuron rather than to actual destruction of receptor-containing neurons. Other animal work shows that differences in HPA activity relate to the variation in density and cortisol-binding affinity of different types of glucocorticoid receptors in the hippocampus (De Kloet, 1991). Specifically, the hypothesis holds that the density of 'type 1' receptors sets the threshold for HPA reactivity, whereas the density of 'type II' receptors modulates the negative feedback. In addition, evidence suggests that previous stress experience alters the number and types of glucocorticoids in the hippocampus.

Although ascertaining an exact physiological mechanism for the higher cortisol, epinephrine and heart rate levels in stunted children is not a focus of this thesis, it is interesting to note the work that has been done looking at mechanism with other forms of malnutrition. Little is known about the mechanisms controlling physiological responses to stressors in undernourished animals, though some evidence suggests that early malnutrition induces enduring changes in central

noradrenergic activity, subsequently altering motivation, emotional reactivity, and cognitive flexibility (Levitsky and Strupp, 1995).

Wasting has recently been shown to be associated with prolonged cortical latency, central motor conduction time, and reduced amplitude of muscle evoked potential, in comparison non-wasted controls (Tamer et al. 1997). The authors suggest that undernutrition may result in dysfunction in cortical threshold maturation. Research with underweight Indian subjects suggests that a low BMI is associated with a generalized increase in β-adrenoceptor responsiveness (Jayarajan et al. 1985; Jayarajan and Shetty, 1987).

Implications of findings

Cortisol and cognitive function

Raised cortisol levels could explain several of the associations of stunting. For example, prolonged glucocorticoid exposure has been associated with hippocampal damage in rats (Xu et al. 1997), primates (Magariños et al. 1996), and humans (Sapolsky, 1996), suggesting a possible link between sustained cortisol exposure and poor cognitive performance in stunted children. Since the hippocampus contains the highest concentrations of glucocorticoid receptors, it is the most vulnerable to damage. Hippocampal neurons that are not destroyed directly by sustained cortisol exposure may have increased vulnerability to, and subsequent death from, hypoxia-ischaemia and exicitoxic seizures and other neurological insults as a result of high glucocorticoid concentrations.

Research with children in St. Vincent evaluated the associations between high cortisol elevations and academic performance for children with and children without inattention/internalizing problems (Durbrow et al. 1998). Interestingly,

cortisol elevations did not seem to inhibit cognitive functioning directly, because the effects of cortisol level on cognitive function appeared only with elevations in cortisol in children who had problems with internalization and inattention. Since the data reported in this thesis show a tendency for stunted children to be more inhibited and less attentive than non-stunted children, it is likely that they may be most similar to the children from St. Vincent who also had problems with inattention.

Growth hormone

Prolonged activation of the stress system also inhibits growth hormone secretion, and several other growth factor effectors (Chrousos and Gold, 1992), implying that stunted children could have experienced blunted growth hormone secretion druing development.

Immunological implications

Psychological stress has been shown to contribute directly to decreased functional immunity, as measured by proliferative response to mitogens and natural killer cell activity (Herbert and Cohen, 1993). Psychological life stress has been shown to be related to increased susceptibility to illness (Cohen et al. 1991; Clark et al. 1996), and duration of illness (Boyce et al. 1977). Even short term laboratory tests of psychological stressors (i.e. mental arithmetic and public speaking) have been shown to increase in circulating natural killer cells, white blood cells, and T lymphocytes, and suppression of PHA-stimulated T-cell proliferation, all of which indicate increased immune activity (Bachen et al. 1992; Cacioppo et al. 1995). Subjects who are induced in a laboratory setting to recall maximally disturbing emotional experiences show significant declines in mitogenic lymphocyte reactivity, with less clear effects on other immune indices (Knapp et al. 1992).

Although the relationship between psychosocial stress, either acute or chronic, and immunity has been well established, it is complicated by individual reactivity, emotional arousal, and perception of the stressor. For example, studies of dental students and West Point cadets have shown an interactive effect of individual emotional state and psychosocial stressors on the incidence of illness; students who have a motivation to achieve but are unable to perform are more likely to develop infectious illnesses (Boyce and Jemerin, 1990). A study of pre-school children showed that the relationship between stress reactivity and incidence of respiratory illness was moderated by level of "life stress" and also by individual tendency for reactivity (Boyce et al. 1995). Namely, children who showed high psycho-biological reactivity to laboratory stress and had low levels of life stress (low-stress childcare or family setting) had lower rates of illness than children who showed low physiological reactivity. Whereas, in situations of high life stress levels, children with high psychobiologic reactivity had higher rates of illness, and children with low psychobiologic reactivity showed no higher illness rates. Thus, children who were the most reactive showed either enhanced or decreased immune function depending on the stress level in the environment.

The connection between psychological stress and dampened immunity may explain the increased rates of infection found in stunted children (Black et al. 1989; Grantham-McGregor et al. 1993), particularly in an urban setting (Worthman and Panter-Brick, 1996). In studies from India, Bangladesh, Papua New Guinea, and Tanzania, wasting has also been shown to potentiate the mortality rate at a given level of disease exposure, with the relative contribution of malnutrition to mortality depending on morbidity rates, and morbidity contribution varying according to malnutrition prevalence (Pelletier et al. 1993). Namely, a synergy exists between

malnutrition and infection implying that the once popular "additive model," trying to quantify the relative contribution of various diseases, has little intrinsic meaning.

Wasting and underweight have been shown to be significant determinants of impaired cellular immune function in Turkana children (Shell-Duncan, 1997). Wasting alone, in Peruvian children, has been associated with incidence, although not duration, of diarrhea (Black et al. 1989); in Bangladeshi children with decreased cellular immune competence, as measured by anergic or not anergic to skin test reactions with three antigens, tuberculin, trichophytin and candidin (Koster et al. 1987).

Cardiovascular implications

The cardiovascular results indicate that stunted children may be at greater cardiovascular risk later in life due to their heightened reactivity (Manuck et al. 1990). The data may also explain the association between adult short stature and enhanced risk of ischemic heart disease (Walker et al. 1989). Chronically high cortisol levels have been associated with risk factors for heart disease, including raised cholesterol levels and endothelial injury (Bell et al. 1993; Troxler et al. 1977).

It is well established that exaggerated psycho-physiological responses to behavioural challenges are risk factors for cardiovascular disease (Murphy et al. 1991; Matthews et al. 1990; Matthews et al. 1986; Krantz and Manuck, 1984), and some investigators have suggested that recurring and excessive cardiovascular responses during periods of stress can lead to the development of high blood pressure (Fredrickson et al. 1991; Paffenbarger, Jr. et al. 1993). Many researchers have shown that shorter body stature is correlated with coronary death in several populations (Paffenbarger, Jr. et al. 1966; Paffenbarger, Jr. and Wing, 1967; Forde and Thelle, 1980; Walker et al. 1989; Coggon et al. 1990; Helmert and Shea, 1997).

Others have only found a relationship in men (Herbert and Cohen, 1993; Rosenberg et al. 1995; Kee et al. 1997) or women (Palmer et al. 1990; D'Avanzo et al. 1994; Kannam et al. 1994; Rich Edwards et al. 1995). However, many researchers have also found no relationship (Rhoads et al. 1978; Greig et al. 1980; Blumchen and Jette, 1992; Rosenberg et al. 1995; Liao et al. 1996; Kee et al. 1997).

Low social class has also been associated with increased risk of heart disease (Gillum and Paffenbarger, Jr. 1978; Notkola et al. 1985; Nyboe et al. 1989; Coggon et al. 1990; Wannamethee et al. 1996; James et al. 1997). Parental history of hypertension results in children with higher stress (mental arithmetic, Stroop color test) responsivity, including exaggerated epinephrine, norepinephrine and cortisol secretion, and more heart rate activity (Fredrickson et al. 1991). These findings indicated that enhanced responsivity was limited only to psychological and not to physical (i.e. isometric exercise) stressors.

Differences in physical fitness between the stunted and non-stunted children may have contributed to the differences in heart rate. Although this hypothesis cannot be validated by the current study design, it could be explored in future studies.

Implications of corporal punishment

In studies from the United States, children whose parents use power assertive child-rearing methods, including physical punishment, are more often rejected by their peers (Hart et al. 1990), have psychological disorders later in life (Wissow and Roter, 1994), and become more aggressive than comparison children (Dodge et al. 1994; Dodge et al. 1990; Sheline et al. 1994; Farrington, 1978; Weiss et al. 1992; Kaufman and Cicchetti, 1989; Olweus, 1994). Although many aggressive behavioural patterns are set in place before children reach school age, their

experiences at school are critical to the development and perpetuation of violent characteristics.

The victims of aggression usually suffer immediate consequences of the bullying, which can include physical and psychological pain and injury. In addition, victims are often repeatedly victimized and are likely to suffer from long term depression (Rigby and Slee, 1991; Olweus, 1994).

A substantial body of scientific literature from developed countries suggests that corporal punishment may have many detrimental effects for children (Stern, 1991; Wissow and Roter, 1994). Students who are physically punished have lowered self-esteem, increased anxiety, fear and depression, poor attention spans, increased aggressive and destructive behaviour, aggression against teachers, vandalism against school property, and deficient academic performance (Rutter, 1980; Walker et al. 1994; Stern, 1991; Poole et al. 1991; Orentlicher, 1992). In addition to these emotional and behavioural consequences of corporal punishment, children suffer from the actual physical consequences, with thousands of children per year needing medical evaluation and/or treatment for abrasions, bruises, broken bones, whiplash, muscle damage, sciatic nerve damage, and brain injury (Poole et al. 1991).

Conclusions from Jamaican studies

The physiological and behavioural findings reported here could have major implications for the millions of growth retarded children worldwide. In two identical studies, with different populations of Jamaican school children, stunted children had higher levels of salivary cortisol and urinary epinephrine, and higher heart rates than non-stunted children, even with the inclusion of several socio-economic variables. Stunted children were also less vocal, less happy, less attentive and more inhibited when compared with non-stunted children. Clearly, in countries where stunting is highly prevalent there is an urgent need to institute programmes to improve the nutritional status of children, given the potential consequences and associations of stunting. These findings need to be replicated in other populations where growth retardation is a common problem.

Chapter 10: Salivary cortisol and heart rate in stunted and non-stunted Nepali school children: a small follow-up study

Introduction

Data from the previously reported Jamaican school children suggests that stunted children have higher concentrations of salivary cortisol and epinephrine, higher heart rates, and altered behaviour when compared with non-stunted children. Given the large percentage of stunted children in the developing world, these findings are highly significant, and could potentially have critical policy implications. However, the findings must be replicated in a population where stunting is more prevalent than it is in Jamaica in order for the interpretation to gain meaning and ecological validity.

The replication study was set in Nepal because 48% of the children under five are moderately or severely stunted, according to UNICEF's State of the World's children, 1999, compared with an estimated 8% of the equivalent Jamaican population. Thus, if the findings could be replicated in a context in which stunting was a much greater public health issue, then the implications for policy would be much greater. Also, it would provide greater insight into the interpretation of findings, and whether stress physiology was related more to nutritional status, cultural issues, or socio-economic facors.

Methodology

Study site: Nepal

Location, climate and topography

Nepal is a small, landlocked country in central Asia bordered by China in the

north and India in the east, south and west (**Figure 21**). With a length of 885

kilometers east-west, and a width varying from 145 to 241 kilometers, Nepal is

147,181 square miles. Nepal is comprised of three major geographical and

topographical areas: forests and cultivatable land in the south, the mighty Himalayas,

including Mt. Everest, in the north, and moderately high mountains in the central

region, which contains the Kathmandu valley and the capital and largest city.

Nepal's climate varies with its topography, ranging from tropical to arctic

depending upon the altitude. The Terai region has a hot humid climate, whereas the

mid-land region is plesant almost all the year round, with cool winter nights. The

northern mountain region, at an altitude of 3,353 m. has an alpine climate with a

considerably lower temperature in winter.

History and racial composition

Nepal, a sovereign independent nation, has a long and glorious history. In

1816, after a war with the British, Nepal adopted a policy of seclusion from foreign

contacts. Internal power struggles led in 1846 to the dominance of the Rana family,

which controlled the country until 1951. Under the Ranas, Nepal was isolated from

foreign influence, and there was little economic modernization. Nepal was granted

independence in 1923, and a limited constitutional monarchy was established in

1951. After a brief period of democracy
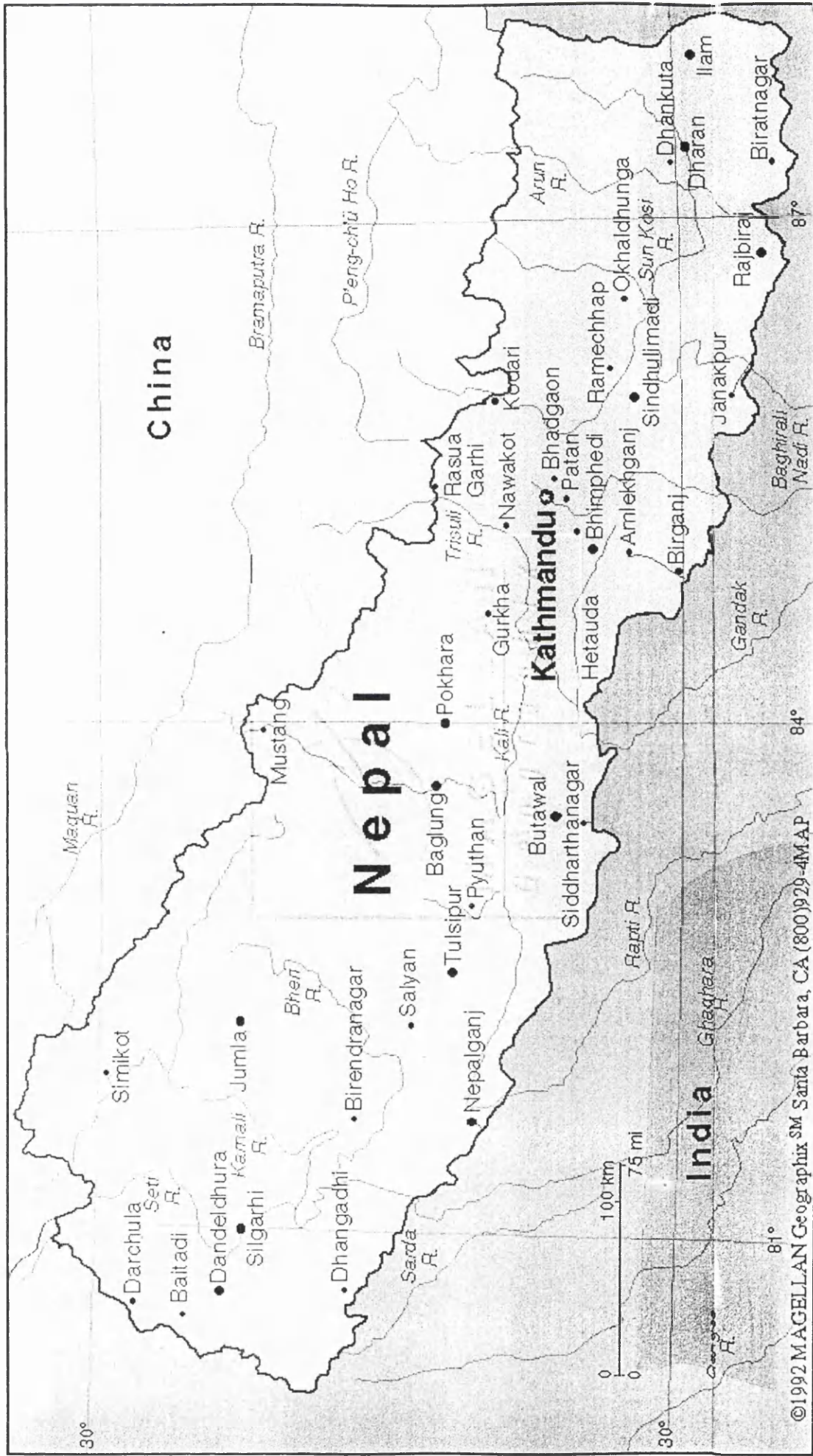
Figure 21: Map of Nepal

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(1959-60), political activity was banned. In elections in 1991 the centrist Nepali Congress Party won a slim majority in the new parliament, and Girija Prasad Koirala became prime minister. Long influenced by India, Nepal has recently developed closer ties with China.

Nepal has a population more than 18 million, who all have different customs and speak different languages and dialects. The Gurungs and Magars live mainly in the west and on the southern slopes of Annapurna, Himalchuli and Ganesh Himal mountains. The Rais, Limbus and Sunuwars inhabit the slopes and valleys of the eastern mid hills. The Newars constitute an important ethnic group in the capital valley Kathmandu and are the most populous in that area, and the Tamangs are very populous in the hills. In the Terai region, there are Tharus, Yadavas, Saar, Rajvanshis and Dhimals. The Brahmans, Chhetri and Thakuris are spread generally over most parts of the Kingdom. Nepali is the official language of Nepal, although English is taught in school from a very early age.

Economic indicators

Nepal's economy is predominantly agricultural, with more than 90% of its population earning a living through cultivation of livestock, sugar, rice, corn, wheat, millet, jute, timber, or potatoes. Nepal has a per capita income of 210 US Dollars.

Public health issues

Nepal has very low levels of economic development, as evidenced by the graphs below which show the infant mortality rates (**Figure 22, Figure 23**) and infections rates for children (**Figure 24, Figure 25**).

Infant mortality rate per 1000 live births

180 160 140 120 100 80 60 40 20 0

164   153   138   122   110   99   89

1965-70   1970-75   1975-80   1980-85   1985-90   1990-95   1995-2000

Year
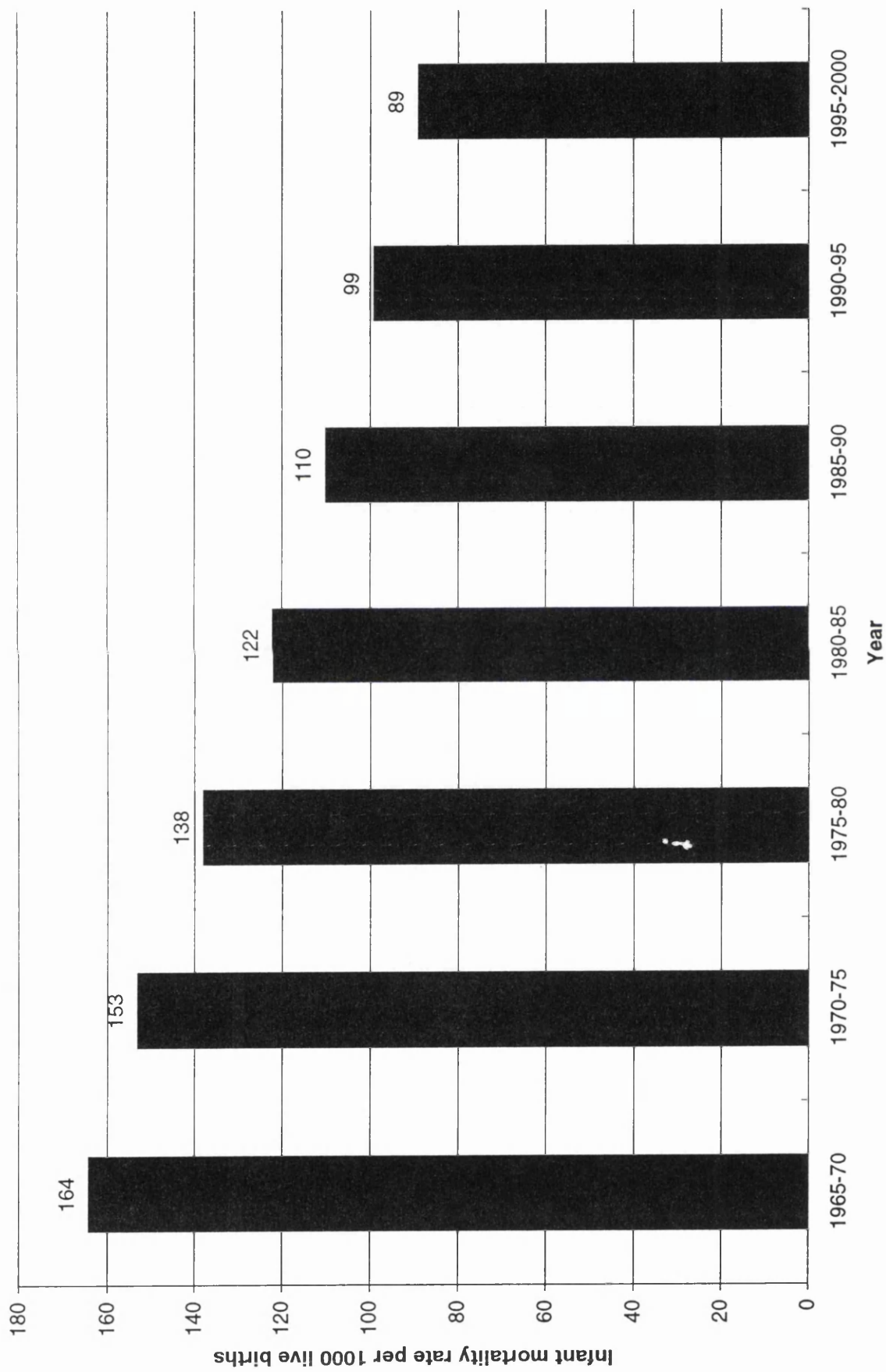
Figure 22: Estimated infant mortality rate per 1000 live births for Nepal from 1965-2000 (values from Health Statistics of Nepal, 1997)
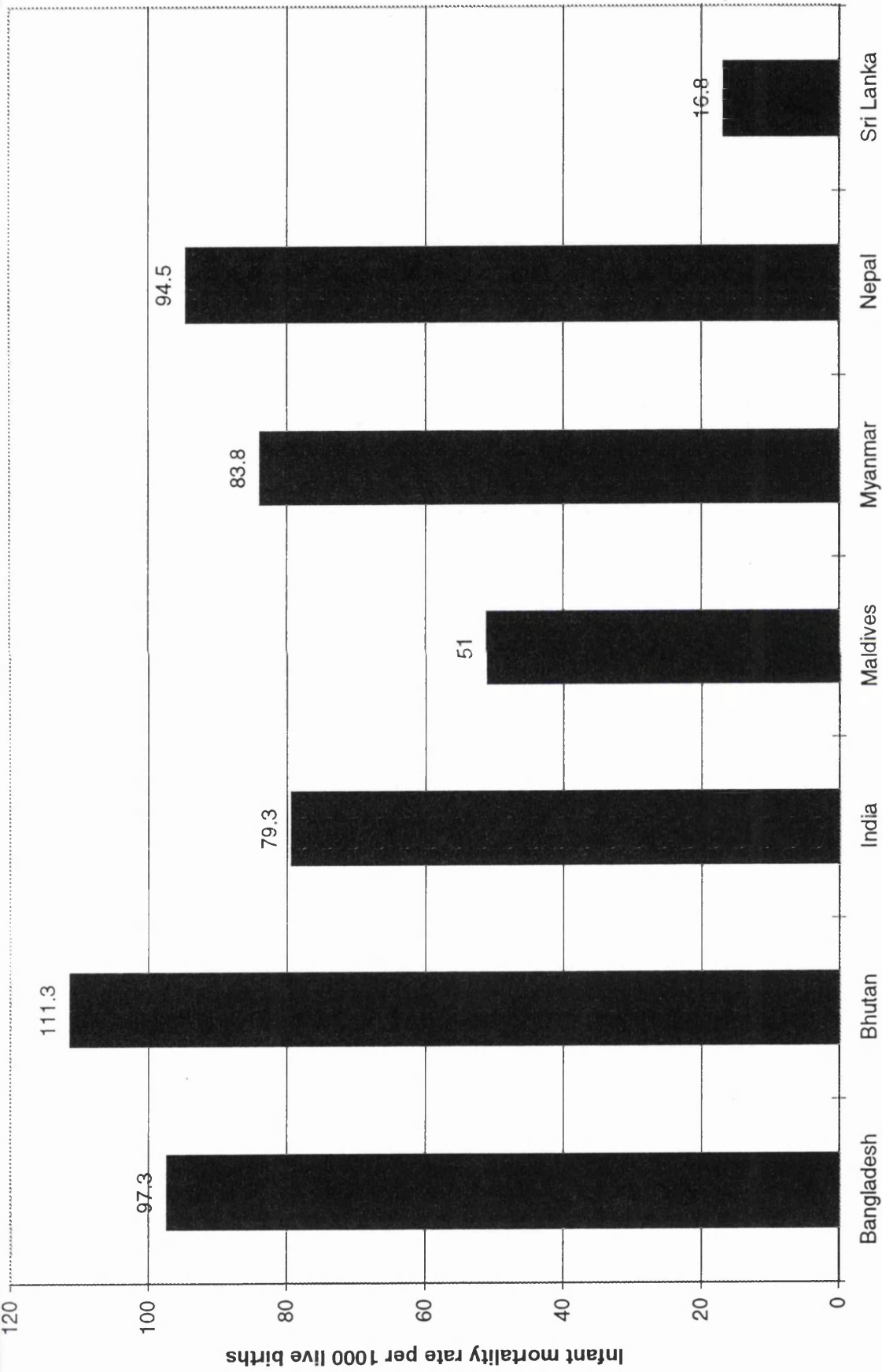
Figure 23: *Estimated infant mortality rates per 1000 live births for SEA (South East Asian) Region for 1991-1995 (mean values from four sources including national data, World Health Report, United Nations and UNICEF, as reported in Health Statistics of Nepal, 1997)*

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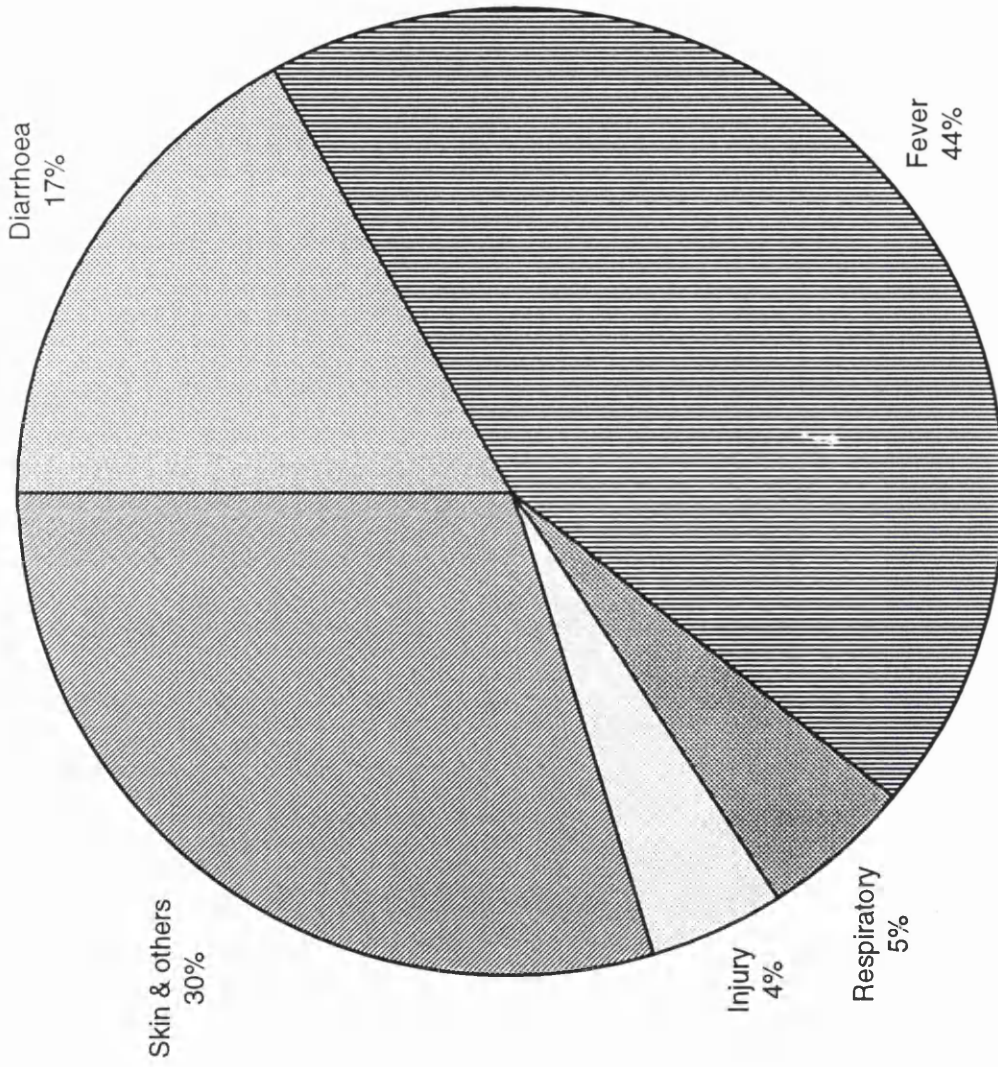
Figure 24: Distribution of type of illness reported for all ages (from Nepal Living Standards Survey Report 1996, as reported in Health Statistics of Nepal, 1997)

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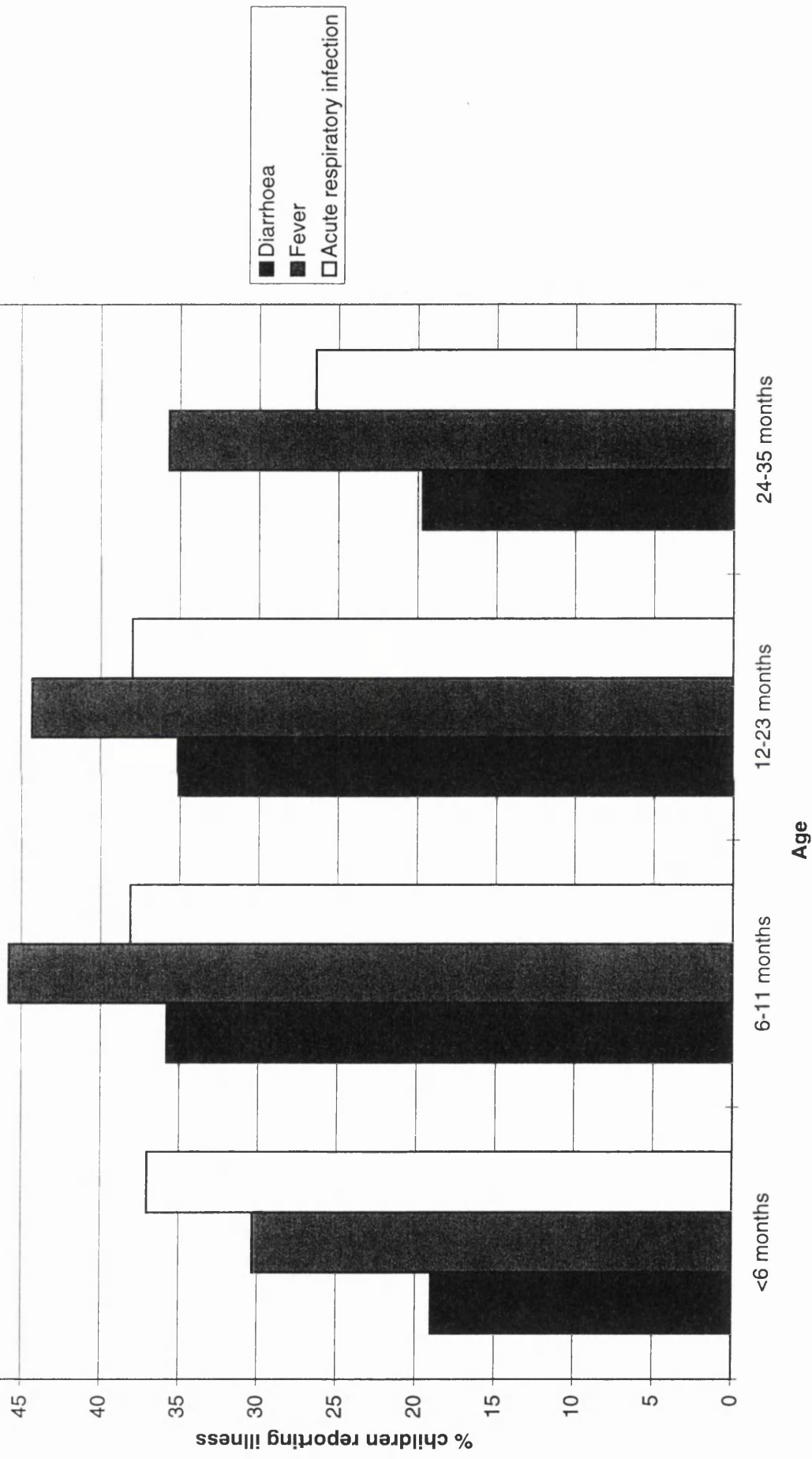
Figure 25: Percentage of children under three years who were ill with diarrhoea, fever or acute respiratory tract infection (described as cough accompanied by fast breathing) during the two weeks preceding the survey in 1996 (data from Nepal Family Health Survey, 1996 as reported in Health Statistics of Nepal, 1997)

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Aims, Objectives and Hypotheses

The goals of the study set in Nepal were:

1) to determine whether Jamaican findings for heart rate and cortisol could be replicated in a population where stunting is much more prevalent.

2) to evaluate early morning salivary cortisol levels and compare them with values on arrival at school in stunted and non-stunted Nepali school children.

The hypotheses being tested was that stunted children would have higher baseline levels and lower reactivity levels (in reponse to a battery of psychological tests) of:

1) Salivary cortisol

2) Heart rate.

Methodology

Sample selection

Nepali school aged (8-10 y.o.) subjects (n=66) and controls (n=64) were identified from 9 schools located in urban areas of the Kathmandu valley, and were all Newari (**Table 28**). Required sample sizes were calculated using data obtained in Jamaica, and were adequate to detect differences greater than 0.5 S.D. between experimental and control groups for the major outcome measures (cortisol and heart rate) as statistically significant at the 0.05 level with a power of 90% (Armitage and Berry, 1994).

Table 28: *Children from urban Kathmandu used for screening and testing*

Name of school	Area	Number of children (number of children tested/ number of children screened)
Shanti Nikunga	Maru	20/48 (42%)
Shree Bhakta Bidhyashram	Raktakali	4/40 (10%)
Prabhat Madhyamik Bidhyalaya	Naghal	22/61 (36%)
Shree Krishna Mandir	Nhyokha	0/37 (0%)
Nawa Adarsha Madhyamik Bidhyalaya	Basantapur	12/17 (71%)
Durbar High School	Ranipokwari	10/38 (26%)
Shaheed Shukra Secondary School	Baag Bazaar	26/51 (55%)
Pragati Shikaya Sadan School	Patan	14/27 (52%)
Kanya Mandir Higher Secondary School	Tengal	20/60 (33%)

All children from each school in grades 1-5 were initially recruited, and requested to obtain proof of birthdate. Every child who produced proof of her/his birthdate from a parent or guardian was measured (**Figure 26**). Then, each child whose height-for-age was less than -2.0 standard deviations from the mean height for age (Hamill et al. 1977) was matched for school, sex and age (± 6 months) with a child whose height for age was greater than -1.0 standard deviations away from the mean. In the case where more than one stunted child matched a non-stunted one for sex and age, then the most stunted one was selected (**Figure 27**). There was no control for ethnicity in the Nepali samples.

Figure 27: Severely stunted child (<-3sd h/a) with age-matched control

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Figure 26: Tester (Kamala Manandhar) screening children at school

To avoid selection bias, the criterion for choosing controls was that they were closest in age to the target subject. If a recruited and eligible child was repeatedly absent from school, s/he was replaced with an alternate, equally eligible child. This replacement technique occurred three times, only in stunted children.

One child was dropped from the study due to miscalculation of height for age at enrollment and subsequent misclassification. Two of the non-stunted control children were unobtainable due to repeated absence from school. Due to the limited number of non-stunted children, no replacements were possible. All subjects were singletons, with no obvious physical or mental handicaps, and housing and maternal education below certain levels. No subject was tested if s/he was physically unwell (i.e. fever, flu or cold).

Ethical approval, in addition to that from the National Institutes of Health and the Institute of Child Health (described earlier), was obtained from the Nepal Health Research Council. Written consent was obtained from parents or guardians, and verbal consent from the teachers, principals and participating children. Great care was taken to ensure that no child was distressed by the testing procedure. In one case, a child was visibly upset before the testing session, for reasons most likely unrelated to the study. She was given a substantial period of time to relax and calm down before any testing occurred. All children who participated in the study were given food, a drink, and 1-2 meters of material with which to make a school uniform. All testing was completed at school (**Figure 28**) by one of four female Nepali testers, each of whom was completely blind to the hypothesis being tested (**Figure 29**).

Figure 28: Typical school yard in Kathmandu
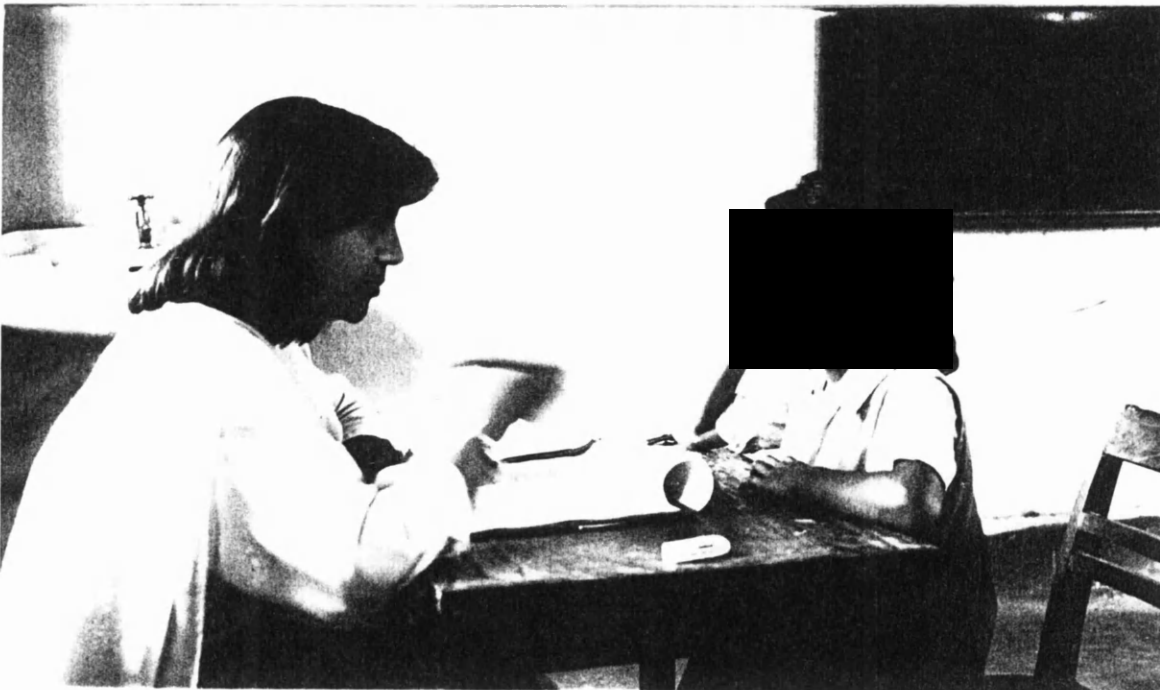
Figure 29: Tester (Sheela Sainju) with child during mental arithmetic

Rationale for choice of stressors

The psychological stressors in the Nepali study were designed to provide a short, intense period of academic stress. Based on the Jamaican results, the mental arithmetic and the Symbols test were most effective in provoking immediate physiological reactivity. A modified Digit Span was also used, which was a modification of a subset of the Wechsler Intelligence Scale for Children that involved immediate recall of increasingly longer strings of digits that were read to the children (Wechsler, 1974).

Outcome measures

<u>Salivary cortisol</u>

Six saliva samples of approximately 0.5 ml each were obtained from each child. The first sample (Sample 1) was a baseline measure and was taken on arrival to the testing room and before the children were given a drink and snack. The second sample (Sample 2) was collected immediately after finishing the psychological stress session (approximately 20 minutes after arrival). The third, fourth and fifth samples were then collected at 10 (Sample 3) , 20 (Sample 4) and 30 (Sample 5) minutes, respectively, after the second sample had been collected. A final sample (Sample 6) was an early morning specimen collected upon wake-up on the morning after testing. The researchers collected the five testing samples, and the parents/guardians were responsible for collecting the early morning sample.

All samples were collected in sterile collection tubes (Salivettes, Sarstedt: Newton, NC) centrifuged briefly (1000g, 2 minutes, 20°C), transferred to cryovials, and then frozen at -20° C. The samples, surrounded by ice packs, were transported back to the lab in the United Kingdom in a cooler. The cortisol levels were evaluated commercially at the University of London using the same (Kirschbaum et

al. 1989) I-125 radio-immuno-assay kit ("Magic Cortisol," Ciba-Corning: Medfield, MA) as the Jamaican saliva, however they were not assayed by the same person.

No significant association existed between the standard pool cortisol reading and the assay (1-12) or the timing of the assay within a run (beginning, middle, end). Intra-asssay correlation (duplicate tubes) was high, ranging from r=0.934 to 0.989; intraassay variation, measured at three times in each of the twelve assays using two standard pools of saliva, was very low, with a coefficient of variation of 9.5% +/- 5.5%. The "response" levels of cortisol (samples 2-5) were moderately inter-correlated ($0.45 < r < 0.58$). To control for the known diurnal rhythm in cortisol levels in the study design, time of testing was balanced across children from both groups. Complete salivary results were available for 127 of 128 children (99%); the child missing a sample did not provide enough saliva for analysis.

Heart rate and haemoglobin

Heart rate was recorded throughout the session via a chest band (Polar Vantage XL heart rate monitor, Polar CIC Inc.: Port Washington, NY) identical to the Jamaican studies. The watches were set to record average heart rates for every 5 seconds. At the beginning and end of each section of testing, the tester pressed a button on the watch, and the exact time was then recorded inside the watch. Using the same technique described for the Jamaican studies, the data from the watch were transferred to computer, and then downloaded to a spreadsheet. Hemoglobin was assessed using the HemoCue (HemoCue Limited: Sheffield, UK), and the technique was standardized using the control cuevette. Full heart rate and hemoglobin results were available for 128 of the 128 children (100%).

Home visits

The mothers or guardians of all children were interviewed either at home or at school. None of the neighborhoods where the children lived had any street names, so it was very difficult to locate a child's home without being directed to the location by someone who knew exactly where it was. Thus, many of the parents/guardians were asked to come to the school after hours for an interview (**Figure 30**). The child was always present for the interview and contributed to the responses, particularly for the questions about the number of toys, books and other materials around the house. In most cases, the children had school bags with them, carrying many of their books.
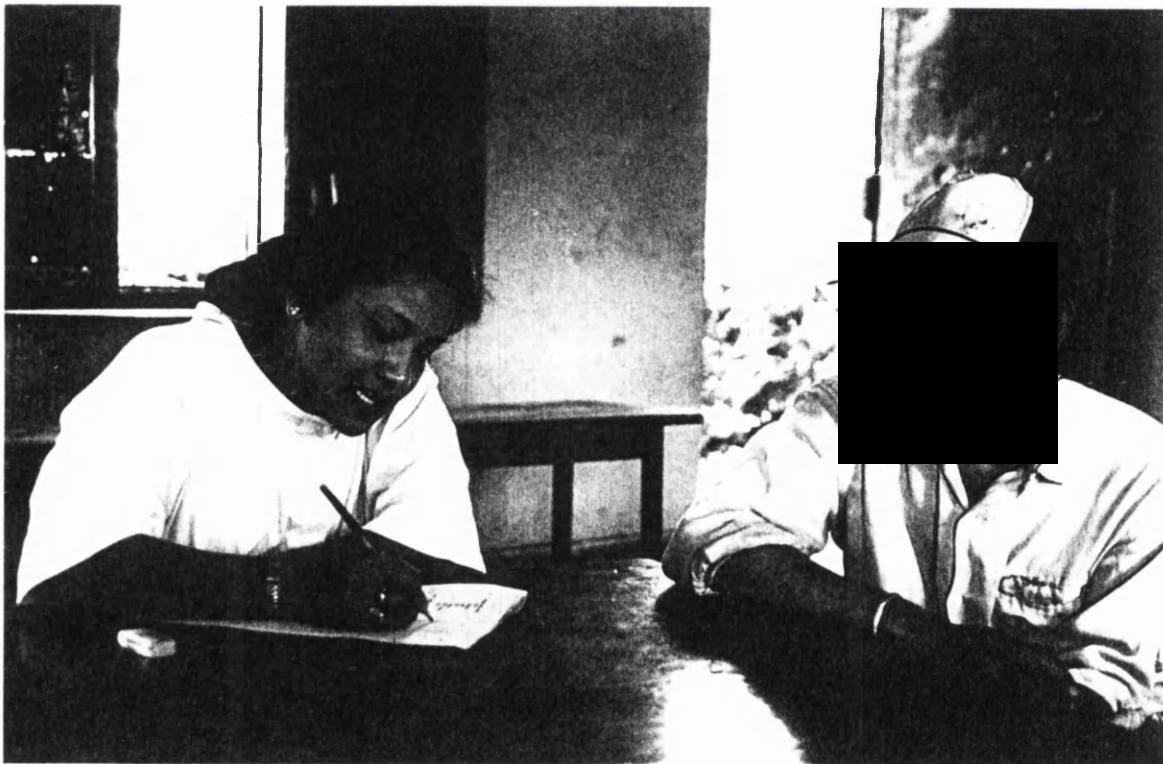
Figure 30: Tester (Ganga Ranjitkar) interviewing child's father

Details of maternal characteristics, including education, occupation, standard of housing, and household possessions were recorded, as was the level of stimulation in the home. The socio-economic assessment in the Nepali study barely differed from the Jamaican. However, during piloting in Nepal, it became clear that some of the questions, particularly about occupation and household composition, needed to be modified to reflect cultural differences. Most of the questions, including those about possessions, crowding and home stimulation remained exactly the same.

The stimulation index included measurements (11 in total) of the number of exercise books, school books, coloring books, paper, pencils & crayons, and toys in the home, and games, trips and verbal stimulation (i.e. reading, telling stories, teaching) from the parents. Other variables were as uncondensed as possible, since the sample size in this study was larger than either of the Jamaican studies. Socio-economic information was available for all children.

Birthweight was requested from mothers or guardians, although most did not have a record of weight. Therefore, a three point rating was used (very small, small, okay). Current weight and height were measured using standard procedures (Lohman et al. 1989). Anthropometric data were available for all children.

General methods

Testing session

The testing session in Nepal was modified in response to the Jamaican experience. The session was shortened substantially, the length of time with psychological stressor was shortened, and the physiological stressors were eliminated (**Table 29**).

Table 29: Mean and standard deviation of events in Nepali testing session in chronological order

Event	Duration[1] (min)
Arrival[2]	
Baseline heart rate (sitting)	5.7 (1.1)
Mental arithmetic	4.1 (1.1)
Symbol Digit Modalities test	3.0 ± 0.8
Digit span test[2]	1.5 ± 0.4
Baseline heart rate (sitting)	5.6 ± 1.0
Departure	

[1] There was approximately 1 minute between events.
[2] Saliva samples were collected at five time points: upon arrival, after completion of digit span test, and 10, 20 and 30 minutes after completion of the digit span test. Time between arrival and baseline (lying) was variable (range 5-20 minutes).

All children were tested at their own schools in downtown Kathmandu, where their salivary cortisols (5 measurements) and heart rates (continuous measurement) were assessed throughout a brief test session of psychological stressors by a female Nepali tester. Hemoglobin was also assessed at the end of the testing session. All children were tested between April and May, 1998, beginning between 8:30 and 13:30; they were matched across time of testing, and there were no significant differences between groups in terms of timing. The entire test session (approximately one hour) included two baseline periods when heart rate was measured, three cognitive function tests and a post-test period when saliva was collected at regular intervals. An early morning sample of saliva was also obtained on the day after testing. Immediately before the first measurement of baseline heart rate (sitting), all children were given an orange-flavored drink and two biscuits (**Appendix IX**) to ensure that they were not hungry during testing, and to equalize

potential postprandial effects. One baseline heart rate period preceded the psychological testing.

Statistical Analysis

Required sample sizes were calculated using data obtained from the Jamaican studies and were adequate to detect differences greater than 0.5 S.D between experimental and control groups for the major outcome measures (cortisol and heart rate) as statistically significant at the 0.05 level with a power of 90% (Armitage and Berry, 1994). Additional subjects were added to account for dropouts. All statistical analyses were two-tailed and performed using a commercial computer program (SPSS 7.1 for Windows: Chicago, IL). Height for age z-scores of the NCHS references were calculated using EpiNut (EpiInfo 6.0, Center for Disease Control: Atlanta, GA).

All mean heart rates for each event were normally distributed. The distribution of salivary cortisol was positively skewed, so the data were log transformed, and were subsequently normally distributed. The transformed data were used in all analyses. Mean "responsive" cortisol was calculated for each subject, as was a mean of the second, third, fourth and fifth measurements, as these were highly correlated. The value for "mean heart rate during psychological testing" was obtained by multiplying the mean values for each event by the duration of the particular event, and dividing by total time. This measure was used to isolate one variable that represented cardiac arousal due to psychological testing.

Bivariate correlations were calculated between the dependent variables and all socio-economic variables, height for age, and birthweight rating with groups separate, and there were no significant associations. After showing that none of the

covariates contributed significantly to any analysis, we repeated the analyses of cortisol and heart rate group differences with birthweight squared as the only covariate.

Residualized gain scores were calculated for both heart rate and cortisol. Linear regression analyses were conducted with the post-test measure (mean value for psychological arousal) as the dependent variable and pre-test measure (base-line value) as the independent variable. The standardized residuals from these analyses were saved as new variables for use as the dependent variables in subsequent multiple regression analyses.

Results

Sample characteristics

Characteristics of the sample of Nepali children are presented in **Table 30**.

Table 30: Mean and standard deviation of sample characteristics of stunted and non-stunted Nepali children

Characteristic[1]	Group		Difference (95% C.I.)	(P)[2]
	Stunted (n=66)	**Non-stunted (n=64)**		
Age (m)	111.0 (7.8)	111.1 (7.6)	0.9 (-2.8, 2.6)	NS
Height for age (z-score)	-2.8 (0.6)	-0.6 (0.6)	-2.2 (-2.4, -2.0)	<0.0001
Body Mass Index (BMI, kg/m^2)	14.9 (1.1)	15.6 (1.4)	-0.7 (-1.1, -0.2)	0.003
Hemoglobin (ug/L)	112.7 (13.6)	116.1 (11.7)	-3.5 (-7.9, 1.0)	NS
Maternal occupation	3.1 (2.3)	2.5 (2.1)	0.6 (-0.2, 0.3)	NS
Paternal occupation	4.4 (1.4)	4.4 (1.6)	0.004 (-0.5, 0.5)	NS
Maternal education	0.7 (1.0)	1.3 (1.7)	-0.6 (-1.1, -0.2)	0.009
Paternal education	2.0 (1.0)	2.4 (1.4)	-0.4 (-0.8, 0.004)	0.05
Stimulation total	8.4 (3.6)	8.0 (3.3)	0.4 (-0.8, 1.6)	NS
Number in household	5.7 (1.4)	6.2 (2.4)	-0.4 (-1.1, 0.2)	NS
Water rating	3.4 (1.0)	3.4 (0.9)	-0.004 (-0.4, 0.3)	NS
Housing rating	10.7 (2.0)	10.7 (3.0)	0.004 (-0.8, 0.9)	NS
Sex (M/F)	34/30	34/30		NS

[1] birthweight is excluded because the data were not valid

[2] P values were calculated using Student's t-test

Differences between the stunted and non-stunted groups in terms of their

socio-economic characteristics were not consistent. Specifically, neither group had a

significantly better socio-economic situation than the other, except for the finding

that father's and mother's education were higher in the non-stunted group. Given

these findings, it is reasonable to assume that the stunted and non-stunted groups

were adequatly matched for socio-economic status.

There was a moderately strong correlation among all the socio-economic

indices, except for number of people living in the household, which suggests that the

measurements were valid as measures of socio-economic status.

Salivary cortisol

Salivary cortisol was positively skewed at all time points, so the data were

logarhythmically transformed, and then had normal distribution. Transformed data

were used in all subsequent analyses. Stunted and non-stunted children did not

differ in terms of salivary cortisol levels (**Figure 31**).

Paired t-test analyses comparing cortisol collected on arrival at school

(sample 1) and cortisol collected on awakening (sample 6) showed no significant

differences, either in the stunted or non-stunted groups (P>0.5 for both), suggesting

that sample 1 could be used as legitimate baseline measure (**Table 31**).
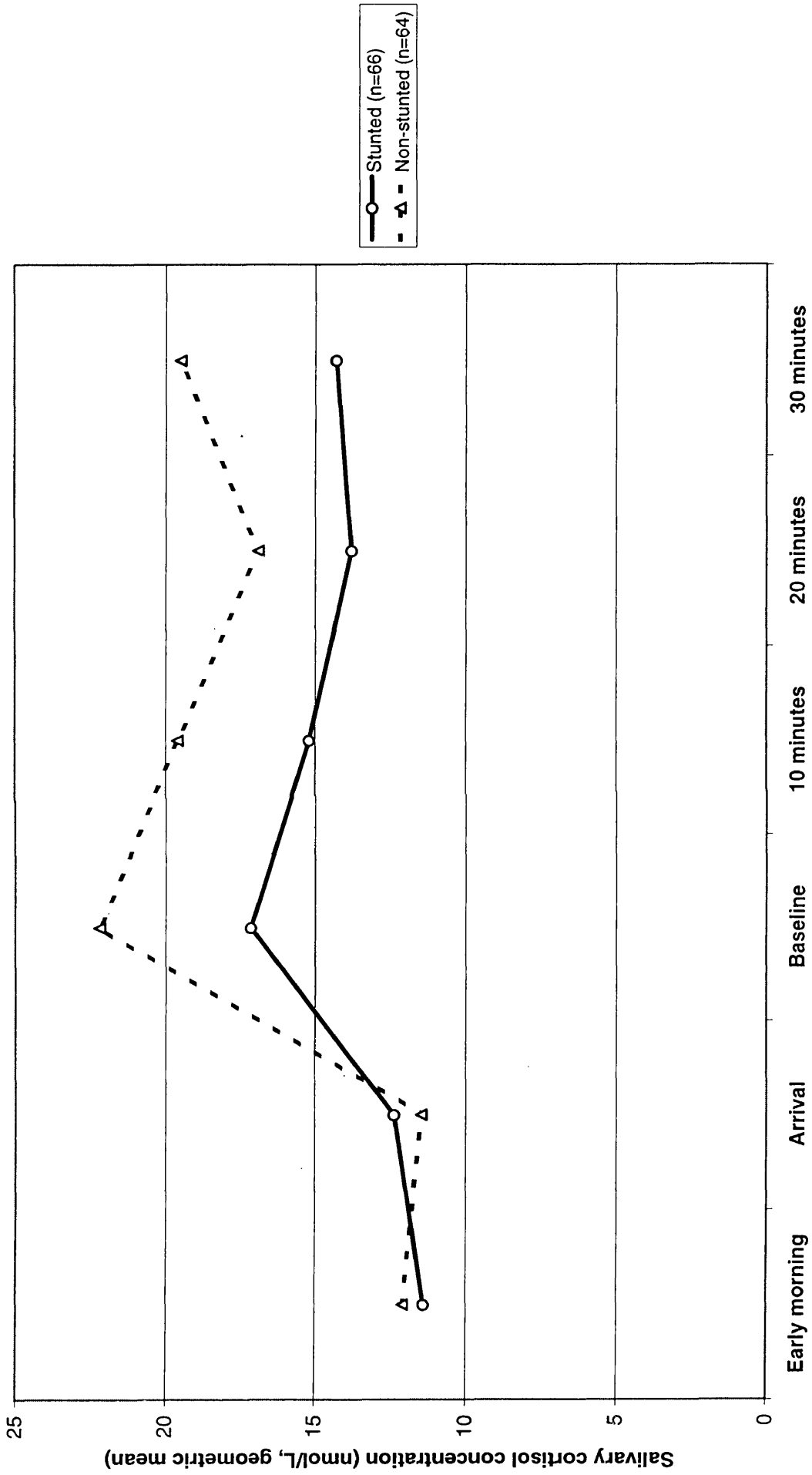
Figure 31: Salivary cortisol concentrations (mmol/L) in stunted and non-stunted children during baseline and psychological testing.

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Table 31: Median and interquartile range for salivary cortisol concentrations (nmol/L) in stunted and non-stunted Nepali children.

Timing of sample	Stunted (n = 64)[1]	Non-stunted (n = 64)
	nmol/L	
Early morning	13.3 (17.0)	13.0 (15.6)
Arrival	11.2 (22.4)	12.4 (17.5)
Baseline	18.1 (23.3)	22.4 (24.1)
Baseline + 10 min	14.7 (24.7)	19.3 (24.4)
Baseline + 20 min	12.8 (30.5)	19.0 (23.1)
Baseline + 30 min	13.7 (17.7)	22.9 (27.8)

[1] n = 63 for arrival sample

Bivariate analyses showed that there were no significant correlations between cortisol level at any point and height, weight, BMI, height-for-age, or any of the socio-economic data collected (education, occupation, housing, water, number in household, or stimulation). These findings held true for the sample as a whole, and for the stunted and non-stunted groups separately. There were also no associations between salivary cortisol and birthweight (small, medium, large), birth order, or gender. For this reason, no socio-economic variables were included in analyses comparing salivary cortisol levels in stunted and non-stunted children.

All children responded adreno-cortically to the test session. It was possible to calculate responsivity by predicting response levels from baseline and group in a linear regression. The response to testing appeared to be diminished in the stunted group, but the difference did not reach statistical significance (P=0.063). There was no interaction between group and test.

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Heart rate

As shown in **Table 32**, heart rates were not different between stunted and non-stunted children. Using the same analysis as with cortisol to evaluate reactivity, the response to the testing appeared to be diminished in the stunted group, but the difference did not reach statistical significance (P=0.18).

Table 32: Mean and standard deviation for mean heart rate (beats/minute) in stunted and non-stunted Nepali children.

Event	Stunted (n = 64)[1]	Non-stunted (n = 64)
	beats / min	
Baseline (sitting)	99.2 (11.6)	100.0 (12.9)
Mental arithmetic	102.2 (13.3)	105.0 (14.7)
Symbols	105.9 (13.2)	109.0 (15.2)
Digit Span	101.5 (13.4)	104.7 (15.1)
Baseline (sitting)	99.2 (12.8)	99.3 (12.7)

[1] n = 62 for Symbols, n = 63 for Digit Span

Cortisol and heart rate together

Both the heart rate and cortisol data suggested that stunted and non-stunted children are identical at baseline, but differ in their responsivity to external stressors. When evaluating heart rate and salivary cortisol together as a combined physiological reactivity score using residualized gain scores to evaluate differences in reactivity while controlling for baseline, it is evident that stunted children have a blunted response pattern when compared with non-stunted children (-0.29 ± 1.37 v. 0.30 ± 1.64, P=0.03). These results mean that an increase in baseline of 1.0 z-score is associated with a decrease in responsivity of -0.29 of a z-score in stunted children, and an increase in responsivity of 0.30 of a z-score in non-stunted children.

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Discussion

This discussion will briefly outline the key findings and will also address some critical issues of validity. Most of the interpretation of the data is discussed in Chapter 11, while also considering the Jamaican findings.

Overview of findings

There were no differences in baseline levels of salivary cortisol or heart rate, when comparing stunted and non-stunted, urban Nepali children. However, stunted children appeared to have a blunted reactivity in response to a short burst of psychological stressors.

The levels of cortisol concentrations found in the Nepali children in this study are high compared with previously reported values for children from the Caribbean (Flinn and England, 1997) and those from Nepal (Panter-Brick et al. 1996a). The heart rates are also somewhat high compared with similar urban school children from Kathmandu, who have a range, while sitting, of 88 ± 9 (Panter-Brick et al. 1996a).

It is unusual to find that stunted and non-stunted children did not differ for any socio-economic variables except education, given the literature about the subject. This finding has two interpretations: 1) the groups were well matched in terms of socio-economic status, 2) the questionnaire did not address the critical socio-economic issues within the cultural context of Nepal.

Validity and limitations of study

The sample size used in Nepal was calculated based on the Jamaican findings, and should thus have had the statistical power to show significant differences between the groups. As is always the case in cross-cultural work,

however, it is not possible to know what effects genetic or cultural differences may have on the outcome variables. Clearly, the best estimates for sample size collection would be based on data collected from very similar children.

The data reported here were analyzed using repeated measures ANOVA, ANCOVA, and multiple regression The assumptions of homogeneity of variance, normal distribution and parallel slopes for the covariage analyses were also satisfied.

To ensure the reliability of the independent variable, selection criteria for subjects were a series of standard, objective measures. The tests were administered in a standard format; all tests in Nepal were performed by four testers who were trained by the same person who tested in Jamaica. Reliabilities were established during piloting and were consistently high. The test session and particularly the timing of saliva collection was highly standardized. The testing record sheet included a checklist to ensure that every step of the protocol was completed.

Testing conditions in Nepal were less standard than in Jamaica because children were tested at school. At each school, children were tested in a vacated classroom with chairs and desks. However, the experiences varied in terms of the number of times a teacher would enter the room, the noise level in the room, and the baseline heart rate experience. Both the stunted and non-stunted groups were confronted with the same range of experiences, suggesting limited effects on the differences between groups. Due to careful matching by sex and school, the study samples were homogenous in terms of age and socio-economic status.

Chapter 11: General discussion, conclusions and suggestions for future research

Introduction

Direct comparisons of absolute values of physiological measurements across studies are unwise, because of large intra-individual variation. Clearly, children born and raised in Kingston, Jamaica and those raised in Kathmandu, Nepal are vastly different in terms of culture, race, health status, and genetic composition. In addition to these fundamental cultural, socio-economic, genetic, health (particularly burden of disease and prevalence of stunting) and demographic differences, there were also substantial differences in the testing procedure, including place of testing (university or school), length of testing (2.5 or 1.5 hours), and components of testing process.

However, it is almost impossible not to want to draw conclusions by comparing these two studies, especially given that the Nepali one was designed initially to be a replication of the Jamaican. Clearly, it is important to be extremely cautious when approaching this issue in order to be as thoughtful and reflective as possible.

Comparisons among studies

One comparison that could be explored is that of the saliva sample on arrival at the testing center (Sample 1 for Jamaicans, Sample 2 for Nepali), since these samples were taken at approximately the same time relative to testing, around the same time of day, and were assayed with the same kits. This comparison indicates

that Nepali children had almost double the "baseline" salivary cortisol levels that the Jamaican children did. The data from Nepali children reported here suggests cortisol measures that are somewhat higher than values previously reported for Nepali children (Worthman and Panter-Brick 1996). Similarly, the baseline heart rates for Nepali children were higher than those measured for Jamaican, and for those previously measured in an urban, Nepali population (Panter-Brick et al. 1996b).

Along with the difference in absolute levels, the Nepali data differ from the Jamaican data in the level of adreno-cortical and heart rate responsivity. The Nepali data show a blunted response in stunted children, for both the cortisol and the heart rate. In contrast, the stunted Jamaican children did not differ in responsivity from the non-stunted children. In the most directly comparable study incorporating a novelty effect (Study 2), non-stunted children appeared to be more reactive than the stunted, but the effect was not significant.

Possible explanations

Reactivity to stressors is a highly individual and contextual process that also reflects a much larger macro-environment. Consistent evidence shows that wide variation exists across individuals who are subject to identical psychological and physiological stressors, with some people responding substantially and others not even registering the event. These differences are most likely to be explained by a constellation of variables. Jamaican and Nepali children come from wildly different backgrounds, which are vary likely to affect how the children experience and respond to psychological and physiological stressors. Gender, age, previous experience, temperament and perceived control have all been suggested as reasons

for differences in HPA reactivity (Nachmias et al. 1996; Flinn and England, 1997; Hart et al. 1995; Kiess et al. 1995; Kirschbaum et al. 1992).

Genetic differences

Animal evidence suggests that genetic factors may also contribute to differences in diurnal cortisol patterns and in cortisol response to stressors (Dhabhar et al. 1993). Racial differences may also have been a factor, given evidence for within-country racial differences in heart rate reactivity (Murphy et al. 1991; Goldstein and Shapiro, 1995; Musante et al. 1995).

Temperament

Another explanation is that Jamaican children did not find the experience as arousing as did the Nepali children. Indeed, anecdotal evidence suggests that the Jamaican children were much more outgoing than the Nepali, and engaged much more readily and comfortably with the testers. In addition, it is very clear from the interviews that Jamaican children must cope with a wide variety of stressors in their day to day lives, and these stressors may be absent from school-children's lives in Kathmandu. Based on these observations, it is altogether possible that the Nepali children were more aroused by the entire experience than were Jamaican children.

Infection rate

A very likely explanation of the very high levels of salivary cortisol in the Nepali children could relate to the burden of disease in these children. Although children who were obviously ill were excluded from the study, no information was collected regarding acute illness or number of days sick in the past year. Studies in the Nepali population have revealed a high prevalence of parasitic infestation and high levels of protein markers of pathogen pressure and inflamation (Worthman and

Panter-Brick, 1996; Panter-Brick et al. 1996) in Nepali children. To investigate this hypothesis, future research should include measures of immune activity, such as immunoglobulin A or interleukin 4.

Percentage of population stunted

Another major difference between Nepali and Jamaican children is the proportion of stunted children compared with the population as a whole is much larger in Nepal than in Jamaica. In Jamaica, children whose heights-for-age are less than two SD's away from the mean comprise a tiny fraction of the population, whereas in Nepal, these children comprise more than half of the population. This difference suggests that stunted children are the norm in Nepal, and are the "outliers" in Jamaica. It may be that children in Jamaica who are severely stunted have experienced some form of psychosocial stress, given how unusual it is for a child to be stunted. This situation differs from that in Nepal, where children are most likely to be stunted due to poor nutrition and/or high levels of infection. Given the wide range of reasons that children can be stunted, discussed in Chapter 3, it is likely that the two populations of children were stunted for different reasons, which could impact their physiological responsiveness.

Socio-economic factors

Although no significant associations between socio-economic measurement and physiological measures emerged during data analysis, all of the children in both studies came from resource-poor environments, which have been hypothesized to be associated with psychosocial stress levels (Flinn and England, 1997). Measurements of economic development like infant survival rates, life expectancy, and GNP per capita are much lower in Nepal than in Jamaica, indicating that urban Nepali

children are fundamentally worse off than their Jamaican counterparts. Thus, the Nepali subjects may have had higher cortisol levels due purely to higher levels of psychosocial or environmental stress. To test this hypothesis, it would be important to try and quantify the actual levels of psycho-social stress in the children's lives, and incorporate those values into the analysis.

Recent work in urban and rural parts of Nepal has shown differences in cortisol level depending on living situation (urban school boys, squatter slum boys, homeless street boys, urban village boys), with the school and homeless children having higher mean levels (Panter-Brick et al. 1996b). Interestingly, it was the village boys who had the highest individual variability in day-to-day cortisol.

In the Nepali study reported in this thesis, the measurements of socio-economic environment were adequate, but by no means sufficient, largely due to time constraints. Ideally, future studies would begin with extensive observation periods in order to determine which factors of the Nepali culture were most important to capture and quantify. The survey used in Nepal was based largely on the Jamaican survey, which had been validated over years of work in that population. However, there was little evidence that the Jamaican study would necessarily be appropriate for Nepal. Although a small pilot study was undertaken in Kathmandu, it was not enough to inspire substantial changes in the Jamaican survey. It would have been very useful to have had a measure of maternal IQ in the Nepali study; this measurement was not completed due to lack of time and lack of validated testing material. Also useful would have been some sort of measure of psycho-social stress, which would have allowed a clearer interpretation of the influence of socio-economic status on the psycho-social home environment.

Birthweight

Birthweight may also have had an impact on the differences in cortisol and heart rate levels between Nepali and Jamaican children, especially given the evidence that children who were light at birth had raised levels of glucocorticoid metabolites in their urinary excretion (Clark et al. 1996). Unfortunately, it was difficult, if not impossible, to obtain accurate birthweight data from Nepali mothers. The closest approximation was to ask the mothers to rate the size that their babies had been at birth, as "small," "medium," or "large," but less than half of the subjects were able to do this. UNICEF reports in the State of the World's Children, 1999 that the prevalence of low birthweight (<2.5kg) in South Asia from 1990-1997 was 33%, whereas the prevalence in Jamaica during the same period was only 10%, which may also explain the higher levels of salivary cortisol in Nepali children.

Summary

It could be that the difference in findings between the Jamaican and Nepali data is due to masking of true results by other external, individual or cultural factors. There may, in fact, be a genuine difference between stunted and non-stunted Nepali children that may have disappeared because baseline levels were pushed very high due to infections, novelty or low birthweight. Given the large inter-individual variability in cortisol levels among the Nepali subjects, it is possible that significant differences would have emerged when using a larger sample size.

Conclusions

When viewed all together, these findings present a dilemma. The Jamaican studies clearly showed higher levels of salivary cortisol, heart rate and urinary epinephrine, and altered behaviour in stunted children as compared with non-stunted

children. The Jamaican studies were carefully designed and controlled, suggesting their strong validity within the urban Jamaican population. The data from the Nepali study show no baseline differences between stunted and non-stunted children, and show that stunted children have blunted responsiveness to psychological stressors, as measured by salivary cortisol and heart rate. Clearly, more work must be undertaken in this critical area, especially given the vast numbers of stunted children throughout the developing world.

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APPENDICES

Appendix I: Map of Kingston

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Record #_____

Date_____/_____/_____
     (mon) (day) (year)

Name_____'_____

Birth date_____

ID Number_____

Time_____

Tester   LF SW

Watch   A B   File   1 2 3 4 5 6 7 8

ANTHROPOMETRY   BREAKFAST

Weight:_____kg   _____

Height:_____cm   _____

First saliva sample, immediately upon arrival _____

First urine sample (20 mintues before beginning of test): _____
Box of milk: _____
Baseline heart rate: _____

Saliva 45 minutes after start: _____

Urine after frustrating task: _____

2nd Baseline heart rate: _____
Saliva at end of 2nd baseline: _____

Weight and height: _____

Checklist before testing:
URINE:
      1) potty clean, and set up
      2) gloves for transfer
      3) funnel
      4) 2 little plastic containers per child

SALIVA:
      1) 3 Salivettes per child

HEART RATE:
      1) HRMonitors have enough time (at least 1:30 per test)
      2) Watch and electrode belt in working condition
      3) Water for back of electrode belt

BASIC EQUIPMENT:
      1) Test sheet, pencil and clipboard
      2) Timer
      3) Two versions of tree/book
      4) Peg board
      5) Beads and thread
      6) Etch-a-sketch, and model drawings

OTHER CONSIDERATIONS
      1) Timer set for 30:00, so that can see 5 second intervals in M.A.
      2) Some food or treat for pegboard
      3) 3 clear beads in container with clogged beads
      4) 2 pieces of equilength, same color pieces of embroidery thread in other container
      5) Gift for child

1. First saliva sample, immediately upon arrival _____
2. First urine sample (20 mintues before beginning of test): _____
3. Box of milk: _____
4. Baseline heart rate for 15 minutes: _____
5. Saliva 35 minutes after start: _____
6. Saliva 50 minutes after start: _____
7. Urine after frustrating task: _____
8. Baseline heart rate for 15 minutes: _____
9. Saliva at end of 2nd baseline: _____
set timer for 35 minutes

A) Interview

After pose question, wait 10 seconds for response.
      •If response, record answer in space provided.
      •If no response after this, then pose question again (be sure to check "repeat" for that
           question). If response, then record. If no response again, then mark "No
           response" as rating.
When child has stopped talking, wait for 10 seconds and then ask "Anything else".
      •If response, record in space provided after "Anything else" prompt.
      •If no response, or shake head, mark 0 with slash through it.

Stop the child if s/he keeps talking after 2 minutes. "Okay, very good then..."

Wait at least 5 seconds between questions

B) Mental Arithmetic
Make sure timer counting down, so can see 5 second intervals

1. INTRODUCTION
   - "Now, we are going to do some maths."
   - "Do you do adding in school?"
   - "Yes?

2. PRACTICE
   - "Okay, let's try 1+1"
   - "What about 1+2?"

(If it seems like the child understands and is giving correct answer, etc..., then go on to diagnostic test. If child not getting it, try 1+1 again, then 2+1, then 1+2, etc. until two in a row correct)

3. DIAGNOSTIC QUESTIONS
Diagnostic questions should be done like test--with 5 seconds per question.

   - All children are tested first with diagnostic test A*.
        => If child gets 0,1,2, or 3 correct out of 5, then do 30 at -A- level
        => If child gets 4 or 5 correct, do diagnostic test B*.
   - For children who do well on A*, test them with diagnostic test B*
        => If get 0 correct, do 30 questions at level -A-
        => if get 1,2, or 3 correct, do 30 questions at level -B-
        => if 4,5 do diagnostic test C*
   - For chidren who reach diagnostic test level C*
        => If get 0 correct, do -B-
        => If get 1,2,3,4,or 5 correct, do -C-

4. TEST
Administer test according to diagnosis.
Preface test with:
   - "Okay, now we are going to have the test."
   - "I want you to answer these questions quick, quick--as fast as you can"
   - "And don't make any mistakes, okay?"

Allow 5 seconds (from when you finish asking question) for them to answer.
   - If incorrect response, say "That's wrong"
   - If out of time, say "No, too slow"
   - If correct, say nothing; just go on to next question

Mark X if wrong, \ if too slow, and check mark if correct.

C) Tree/book

Use only the book/pencil forms

1. INTRODUCTION--*USE FORM A*
   - •"See the square, see the funny shape?"
   - •"What's inside the _____?"
   - •"Right, that's a book inside the square and a pencil inside the funny shape."
   - •"Now, when you see a square, I want you to say book, and when you see a funny shape, I want you to say pencil, okay?

2. SHOW ONE LINE
   - •"So here, this would be _____, next, next, next,..."

3. PRACTICE THREE LINES
   - •"Now try these 3 lines (run finger along 3 lines from left to right)
   - •"Use your own finger, and touch each one as you say it."
   - •"Try to get them all right"

=>If more than 3 wrong, then try to do the next 3 lines.
   "Now, try and do these next three lines as quickly as you can, and try not to get any wrong."

=>If still seems like they don't understand, then do last 1-2 lines.

4. TEST 1: *USE FORM B*
   - •"Now, I want you to do this whole page. Ready. Go. Quick, quick."
       Start timer on "Go" and record time per page and errors per page.
   - •"Quick, quick" at the beginning of every third line.
   - •Stop timer when child makes last stroke

5. TEST 2: *USE FORM C*
   same instructions

D) Pegboard

1. INTRODUCTION
   - •"See this little thing shaped like a key?
   - •"It goes in here just like this--see?"
       (Do two to illustrate concept, then let them finish line)
2. PRACTICE
   - •"Now, you try to do these next two lines"
   (If after doing 3 from top line, and two entire rows, child does not seem to be understanding, then try another two. Do as many lines as required for child to understand. The idea is to eliminate the practice effect.)

3. TEST
   - •Now I want you to do these three lines as quickly as you can"
   - •"Quick, quick" on each third line.

E) Frustrating task

BEADS
Each child is presented with 2 plastic containers. In one are 2 pieces of embroidery thread (identical length and color), and in the other are 20 beads of different colors (equal color distribution). There should be three beads clogged with glue in the bead bowl.

1. INTRODUCTION
- •"See these pretty beads here?"
- •"See this nice thread here?"
- •"I want you to put as many of these beads (pick one up and show for emphasis), onto one of these threads (pick up)." (make sure they understand concept)
- •"You can keep as many of the beads as you put onto the string."
- •"You only have 10 minutes, so work quickly"

2. EXERCISE
- •"Ready? Okay--here's one for you to start with" (give them a clear bead)
- •Start timer and start watching!

ETCH-A-SKETCH
1. INTRODUCTION
- •"See this here--it's something you can use to make pictures."
- •"Now, this knob here you can use to make lines from side to side" (demonstrate)
- •"Now, this knob here you can use to make lines go up and down." (demonstrate)
- •"See how that works?"
- •with much emphasis: "To make lines that are curvy, or crooked, then use both knobs at the same time" (demonstrate)

2. PRACTICE
- • "Now that you understand what these knobs do, I want you to try and make a square--try and make it look like this." (point to picture)
- •"Okay, very good. Now, I want you to try and draw this circle."
- •"Remember, to make curved lines, you use both knobs at this same time."
- •"You only have 5 minutes, and I want it to look good, okay?"

3. EXERCISE (start timer for 5 minutes)
- •"Ready? Okay, go!"

RATINGS

Vocalizations
    A. Doubting competence ("can't do it" "mi spoil it")
    B. Task related explanation of failure ("won't come now" "this can't go in")
    C. Requesting assistance or information ("show me" "help me" "you do it")
    D. Exasperation ("tcha", mumbling, grumbling, sighing)
    E. Other task related vocalizations
    F. Other non-task related vocalizations

Movement
    1-Normal movement, only related to task.
    2-Some extraneous movement evident
    3-Moderately fidgety, obvious, but < 1/2 time
    4-Small mvmnts all time, or big 50-75% time

5-Extraneous movement >75%

Signs of Frustration

SIGNS OF FRUSTRATION:
1 -no signs of frustration;
2 -some signs, few, scattered;
3-somewhat frustrated, <50%;
4-several signs, 50-75%;
5-many signs of exasperation, more than 75%.

==> looking up at tester, signs of exasperation, grimicing, sighing, shrugging

Attention

1-Easily distracted, looks up at any provocation
2-Mildly engaged, but fairly easily distracted
3-Somewhat engaged, occassionally distracted
4-Clearly engaged, occupied, almost never distracted
5-Intensely absorbed, engaged, concentrating

Time on task

In first 5 minutes (beads), or first 2.5 minutes (E-S):
•they stop and declare "I'm finished"
=>wait 30 seconds, and then say "try again"
•they stop without saying anything
=>after 30 seconds you ask "Are you finished"
if "yes" then you say "keep trying".
if "no", then presumably they will keep trying.
In second half of time:
•they stop and declare "I'm finished" then take away immediately

Take urine sample after frustrating task!!

F) Handgrip:

•Set at 30, have them squeeze as hard as can.
•Multiply by 3 to get value for 30%
•point pencil and tell them to sustain for 2 minutes.

Appendix III: Testing sheet

NAME:_____ ID:_____ DATE:_____ RECORD #:_____

A) INTERVIEW

Time start interview: _____

I'm going to ask you some questions, okay?

1. Can you tell me what happens at school? (repeat?___)

_____
_____
_____
_____

Anything else? (shake head or indicate no ___) _____
_____

____1-No Response, or "nothing"
____2-Single words, not joined together in sentences, or, 1 three or four word sentence
____3-Less than or equal to three incomplete sentences, or, less than or equal to three short complete sentences, or, one long sentence with three or less verbs.
____4-Two to four longer sentences, or, greater than three short sentences, or, one long sentence with more than three verbs
____5-More than or equal to 5 longer sentences, with details

Level of inhibition
____1 Very withdrawn (avoiding eye contact with tester, eye contact that occurs is fleeting, and infrequent (0-2x), clearly very uncomfortable)
____2 Fairly withdrawn (fairly uncomfortable, more than fleeting eye contact, but less than half of the session)
____3 Neither withdrawn nor outgoing (substantial eye contact (more than half the time))
____4 Fairly outgoing (eye contact most of the time, fairly comfortable)
____5 Very outgoing (eye contact all of the time, with few lapses, clearly comfortable)

Movement
____1 No fidgeting, absolute stillness, barely any movement
____2 Minimal movement (clearly still, but not completely frozen)
____3 Normal movement, no "unusual" behavior
____4 Some fidgeting (obvious movement, but less than 75% the time)
____5 Lots of fidgeting* (small movements all the time, or big movements more than 75%)

*Fidgeting defined as fiddling with clothes or face, pulling hair, hands touching face, sucking on fingers, chewing on pencil, shuffling feet.

Happy Affect
____1 Clearly unhappy, not enjoying situation, upset, anxious
____2 Somewhat unhappy, occassional furrowed brow or grimace
____3 Neither unhappy nor happy, exhibiting characteristics of neither
____4 Somewhat happy, occassional smile, grin, or laugh
____5 Clearly happy, smiling, enjoying situation

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2. Can you tell me, what does a teacher do?   (repeat?___)

_____

_____

_____

_____

_____

Anything else? (shake head or indicate no ___)   _____

_____

___1-No Response, or "nothing"
___2-Single words, not joined together in sentences, or, 1 three or four word sentence
___3-Less than or equal to three incomplete sentences, or, less than or equal to three short complete sentences, or, one long sentence with three or less verbs.
___4-Two to four longer sentences, or, greater than three short sentences, or, one long sentence with more than three verbs
___5-More than or equal to 5 longer sentences, with details

3. Can you tell me, what does a principal do?   (repeat?___)

_____

_____

_____

_____

_____

Anything else? (shake head or indicate no ___)   _____

_____

___1-No Response, or "nothing"
___2-Single words, not joined together in sentences, or, 1 three or four word sentence
___3-Less than or equal to three incomplete sentences, or, less than or equal to three short complete sentences, or, one long sentence with three or less verbs.
___4-Two to four longer sentences, or, greater than three short sentences, or, one long sentence with more than three verbs
___5-More than or equal to 5 longer sentences, with details

4. Can you tell me, what does a father do?   (repeat?___)

_____

_____

_____

_____

Anything else? (shake head or indicate no ___)   _____

_____

___1-No Response, or "nothing"
___2-Single words, not joined together in sentences, or, 1 three or four word sentence
___3-Less than or equal to three incomplete sentences, or, less than or equal to three short complete sentences, or, one long sentence with three or less verbs.
___4-Two to four longer sentences, or, greater than three short sentences, or, one long sentence with more than three verbs
___5-More than or equal to 5 longer sentences, with details

5. Tell me, what does a mother do?   (repeat?___)

_____
_____
_____
_____
_____

Anything else? (shake head or indicate no ___) _____

_____

___1-No Response, or "nothing"
___2-Single words, not joined together in sentences, or, 1 three or four word sentence
___3-Less than or equal to three incomplete sentences, or, less than or equal to three short complete sentences, or, one long sentence with three or less verbs.
___4-Two to four longer sentences, or, greater than three short sentences, or, one long sentence with more than three verbs
___5-More than or equal to 5 longer sentences, with details

6. Tell me, what does a policeman do?   (repeat?___)

_____
_____
_____
_____
_____

Anything else? (shake head or indicate no ___) _____

_____

___1-No Response, or "nothing"
___2-Single words, not joined together in sentences, or, 1 three or four word sentence
___3-Less than or equal to three incomplete sentences, or, less than or equal to three short complete sentences, or, one long sentence with three or less verbs.
___4-Two to four longer sentences, or, greater than three short sentences, or, one long sentence with more than three verbs
___5-More than or equal to 5 longer sentences, with details

7. Tell me what happens if you lose your lunch money.   (repeat?___)

_____
_____
_____
_____
_____

Anything else? (shake head or indicate no ___) _____

_____

___1-No Response, or "nothing"
___2-Single words, not joined together in sentences, or, 1 three or four word sentence
___3-Less than or equal to three incomplete sentences, or, less than or equal to three short complete sentences, or, one long sentence with three or less verbs.
___4-Two to four longer sentences, or, greater than three short sentences, or, one long sentence with more than three verbs
___5-More than or equal to 5 longer sentences, with details

8. Tell me what happens if you lose your school book that your mother gave you. (repeat?___)

_____

_____

_____

_____

Anything else? (shake head or indicate no ____) _____

_____

____1-No Response, or "nothing"
____2-Single words, not joined together in sentences, or, 1 three or four word sentence
____3-Less than or equal to three incomplete sentences, or, less than or equal to three short complete sentences, or one long sentence with three or less verbs.
____4-Two to four longer sentences, or, greater than three short sentences, or, one long sentence with more than three verbs
____5-More than or equal to 5 longer sentences, with details

9. Tell me what happens if you tear your uniform. (repeat?___)

_____

_____

_____

_____

Anything else? (shake head or indicate no ____) _____

_____

____1-No Response, or "nothing"
____2-Single words, not joined together in sentences, or, 1 three or four word sentence
____3-Less than or equal to three incomplete sentences, or, less than or equal to three short complete sentences, or one long sentence with three or less verbs.
____4-Two to four longer sentences, or, greater than three short sentences, or, one long sentence with more than three verbs
____5-More than or equal to 5 longer sentences, with details

10. Tell me what happens if you're late to school. (repeat?___)

_____

_____

_____

_____

Anything else? (shake head or indicate no ____) _____

_____

____1-No Response, or "nothing"
____2-Single words, not joined together in sentences, or, 1 three or four word sentence
____3-Less than or equal to three incomplete sentences, or, less than or equal to three short complete sentences, or one long sentence with three or less verbs.
____4-Two to four longer sentences, or, greater than three short sentences, or, one long sentence with more than three verbs
____5-More than or equal to 5 longer sentences, with details

11. Tell me what happens if you get in trouble at home.   (repeat?___)

_____

_____

_____

_____

Anything else? (shake head or indicate no ___)   _____

_____

___1-No Response, or "nothing"

___2-Single words, not joined together in sentences, or, 1 three or four word sentence

___3-Less than or equal to 3 incomplete sentences, or, less than or equal to 3 short complete sentences, or,
       1 long sentence with 3 or less verbs.

___4-2-4 longer sentences, or, greater than 3 short sentences, or, 1 long sentence with more than 3 verbs

___5-More than or equal to 5 longer sentences, with details

12. Tell me what happens if someone that you know from your class tief your money. (repeat?___)

_____

_____

_____

_____

Anything else? (shake head or indicate no ___)   _____

_____

___1-No Response, or "nothing"

___2-Single words, not joined together in sentences, or, 1 three or four word sentence

___3-Less than or equal to 3 incomplete sentences, or, less than or equal to 3 short complete sentences, or,
       1 long sentence with 3 or less verbs.

___4-2-4 longer sentences, or, greater than 3 short sentences, or, 1 long sentence with more than 3 verbs

___5-More than or equal to 5 longer sentences, with details

Questions 2-12

___1 Very withdrawn (avoiding eye contact with tester, eye contact that occurs is fleeting, and infrequent (0-2x),
       clearly very uncomfortable)

___2 Fairly withdrawn (fairly uncomfortable, more than fleeting eye contact, but less than half of the session)

___3 Neither withdrawn nor outgoing (substantial eye contact (more than half the time))

___4 Fairly outgoing (eye contact most of the time, fairly comfortable)

___5 Very outgoing (eye contact all of the time, with few lapses, clearly comfortable)

Movement

___1 No fidgeting, absolute stillness, barely any movement

___2 Minimal movement (clearly still, but not completely frozen)

___3 Normal movement, no "unusual" behavior

___4 Some fidgeting (obvious movement, but less than 75% the time)

___5 Lots of fidgeting* (small movements all the time, or big movements more than 75%)

*Fidgeting: fiddling Clothes/ face, pulling hair, hands/ face, sucking on fingers, chewing on pencil, shuffling feet.

Happy Affect

___1 Clearly unhappy, not enjoying situation, upset, anxious

___2 Somewhat unhappy, occasional furrowed brow or grimace

___3 Neither unhappy nor happy, exhibiting characteristics of neither

___4 Somewhat happy, occasional smile, grin, or laugh

___5 Clearly happy, smiling, enjoying situation   Time finish interview: _____

C) TREE/BOOK

	A	B
Time start:	_____	_____
Time finish:	_____	_____
Number wrong:	_____	_____

SAY "QUICK, QUICK" EVERY THIRD LINE

D) PEGBOARD

	A	B
	WITHOUT FOOD	WITH FOOD _____
		WITHOUT FOOD _____
Time start:	_____	_____
Time finish:	_____	_____
Elapsed time	_____	_____

SAY "QUICK, QUICK" EVERY LINE

E) FRUSTRATING TASKS

*****take urine sample after frustrating task, then come back for handgrip******

F) HANDGRIP

Set at: _____(if 30, check here_____)

Maximum: _____

Sustain at _____ (multiply maximum by 3)

Time start: _____

Time finish: _____

VOCALIZATIONS / STRATEGIES

	SELF DOUBT	TASK FAIL.	HELP/ INFO	EXASP	OTHR. TASK	NON-TASK	LICK/ SUCK	DIVIDE	TWIST	SMOOTH	CHANGE ENDS	CHANGE THREAD	CLEAR BEAD	LOOK IN BEAD	USE BOWL	#X CHG BEAD
START TIME:																
END:																
TIME LEFT:																
TOTAL																
MVMT.																
FRUST.							#TIMES SHAKE									
ATTEN.																
HAPPY																
TIME START:																
END:																
LEFT:																
TOTAL																
MVMT.																
FRUST.																
ATTEN.																
HAPPY																
NOTES:																

Ratings:

MOVEMENT: 1-normal movement, 2-some minimal extraneous, 3-moderately fidgety. less than 1/2 the time, 4-small all the time, or big 50-75% of time, 5-mvt >75%. ==>shuffling, stomping feet; moving back and forth in chair; hand in hair; squirming: changing pos. of activity on table; tapping fingers, etc.

SIGNS OF FRUSTRATION: 1-no signs of frust.; 2-some signs, few, scattered; 3-somewhat frustrated, <50%; 4-several signs, 50-75%; 5-many signs of exasperation, more than 75%. ==> looking up at tester, signs of exasperation, grimicing, sighing, shrugging

ATTENTION: 1-easily distracted, looks up at any provocation, and even without provocation, jumpy, not engaged in task; 2-mildly engaged in task, but fairly easily distracted when provoked; 3-somewhat engaged, occassionally distracted; 4-clearly engaged, occupied, almost never distracted; 5-intensely absorbed, engaged concentrating.

HAPPY AFFECT: 1-clearly unhappy, upset, anxious; 2-somewhat unhappy, furrowed brow etc. 3-neither happy nor unhappy; 4-somewhat happy, occassional smile; 5-clearly enjoying situation, smiling frequently; laughing.
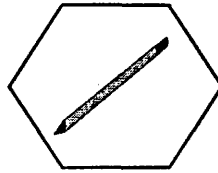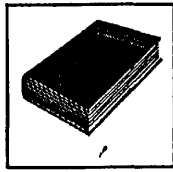
1. Thaw samples overnight in refrigerator. Transfer samples and pools to ice.

2. Bring standards, tracer (shielded), antibody and PBS to room temperature.

3. Record date of preparation of standards, tracer, PBS. If there is less than 200 ul left of any standard, prepare new standards and test them by running them at the end of the assay. To prepare standards, mix together 100ul of each Ciba standard with 900 ul of PBS to make final concentrations of 0.0, 0.1, 0.25, 0.6, 1.5, 3.5, and 7.5 ug/dl. vortex for 30 seconds

4. Write up assay plan (tube numbers, contents, comments). Remember that duplicates must always start with an odd number for the counter to align them correctly.

5. Label all tubes with tube number.

6. Tubes 1 and 2 are "total" containing 50ul of tracer only. After pipetting these two tubes, you can parafilm them and set them aside in a different test tube holder -- they do not go into the magnetic separator or get decanted like the rest of the tubes.

7. Tubes 3 and 4 are "blanks" containing 100 ul of PBS (instead of antibody), 90 ul of the 0.0 standard, and 50 ul of tracer. You might want to put parafilm on these two tubes to remind yourself not to add antibody. These do get decanted with the rest of the assay.

8. Vortex each sample, standard or control for 30 seconds immediately prior to pipetting (set vortexer to level 4.5). Use a new tip for each sample.

Pipette 90ul of standards (using P200), pool controls, and samples into the bottom of each tube.

9. Mix antibody on shaker for 10 minutes prior to pipetting. Also shake the bottle prior to removing each new aliquot.

10. Pipette 100 ul of antibody into the bottom of each tube using the 5 ml reservoir tip with the repeat pipetter (yellow tip). Between tubes, wipe the drop on the tip of the pipette off (using a kimwipe wrapped around your finger) or, if the drop is left on the side of the tube, wipe it off with a q-tip. (Be careful not to splash the antibody into the tube).

11. Swirl the bottle of tracer a bit before pipetting 50 ul of tracer into the bottom of each tube (using a 2.5 ml reservoir tip and the repeat pipette: fill reservoir, press once, wipe off with kimwipe). For best accuracy, touch the pipette tip to the side of each tube after depositing tracer (leaves a drop at the top). Then go back and remove all drops with q-tips. The tracer drops are usually too big to wipe them on a kimwipe because they fall off. Pipette extra back into bottle.

12. Incubate all tubes at room temperature for 3.5 hours after covering with parafilm. Start timing the incubation after you've added all tracer to the first rack.

13. Put all refrigerated items back into the cold room.

After 3.5 hours...

14. Attach magnetic bases to each rack. Let the tubes sit while magnetic separators work for 5 minutes.

15. Wearing glasses and magnetic shield: with the magnets attached, decant the tubes into a pan. Use rocking motions to shake excess drops from the tubes.

16. Rinse each tube with 1 ml of distilled water (using unlabeled eppendorf, 50 ml). Deposit the water at the top, and let the water run down the side of the tube. Let the tubes sit for 5 more minutes.

17. With the magnets attached, decant the tubes into a pan once again. Using rocking motions once again, and then let the tubes drain onto soaker paper for 5 minutes. q-tip extra water to 1/2 inch from bottom of tube.

18. Dispose of the liquid waste into the cement can and rinse the tray with 25 ml of distilled water. Wipe the tray clean with one paper towel (dispose of this paper towel in the dry radioactive waste bin).

19. Take the tubes to the counter in the magnetic racks. Transfer the tubes to the counter racks and proceed to count.

20. Make sure you have recorded the amount of radioactive waste created and how much radioactive material was used from the tracer bottle (~90% liquid, ~10% dry).

Principle

After addition of internal standard (dihydroxybenzylamine) to urine, catecholamines are isolated by ion exchange chromotography on Biorex 70 at pH 6.5, according to the method of Odink et al. 1986 (ODINK1986).

Equipment

Hewlett Packard series 1050 isocratic pump

Hewlett Packard series 1049 Programmable electrochemical detector

Hewlett Packard 3396 series II integrator

Mobile Phase

3.84 g citric acid, 0.1 g EDTA and 0.3g octanesulphonic acid made up in 900 ml water. After pH adjustments to 5 with 2N NaOH, 100 ml methanol is added, and the mobile phase is filtered through a 0.45 μm filter.

Column

Merck Lichrosphere 100 RP-18, 5 μm with guard column.

Comments on sample preparation

Sample purification was performed on 0.6 g Biorex 70 resin (Biorad) after addition of 0.1% EDTA to urine, addition of Internal Standard and adjustment of sample pH to 5-6.5. After washing columns with water, elution of the boronate complexes was performe with 2% w/v boric acid.

The standards (Sigma) were made up in 0.1N Hcl (stock standards) and further diluted in 2% boric acid prior to chromotography. The addition of boric acid was found to be necessary, since the chromatographic properties (i.e. retention times) for free catecholamines and catecholamine boronate complexes are different.

Peak height was used for calculation of results. A linear relationship between peak height and concentration over a wide range has been established.

Appendix VII: Home questionnaire

Student No: _____   Class No: _____   Interviewer: _____

Student's name: _____   _____

Date of birth: _____   Age (m): _____ Sex: M(1)   F(2)

Address: ____ _____   _____

Person interviewed & relationship to child: _____

A. FAMILY INFORMATION

	FATHER	MOTHER
1. Name		
2. Education		
Illiterate	(0)	0
Primary	1	1
Secondary	2	2
College/university	3	3
3. Occupation		
None	0	0
Farmer	1	1
Daily wage earner	(2)	2
Salaried--low	3	(3)
Salaried--high	4	4
Business--small	5	5
Business--big	6	6
Others (99)		

4. Number of children born to mother: _____

5. Birthorder of student: _____

6. Number of people: a. under 5 years old   _____
                       b. 14 years or less   _____
                       c. 15 years or more   _____

7. Total living in household   _____

B. HOUSING INFORMATION

8. Do you rent or own your home?   rent   _____ (0)
                                     own   _____ (1)

9. How many rooms does your family live in?   _____

10. Housing type		11. Water supply	
Zinc sheets, cardboard	0	Outside well, far from house	0
Mud	1	Outside well, near to house	1
Raw bricks	2	Shared pipe near to house	2
"Cooked brick"	3	Hand pump inside house	3
Cemented	④	Piped inside house	④
12. Toilet		13. Cooking	
No toilet	0	Firewood	0
Share non-flushed	1	Charcoal	1
Have own non-flushed	②	Kerosene/oil stove	②
Share flushed	3	Gas stove	3
Have own flushed	4	Electric stove	4

14. Possessions

	No	Yes	
Stove	0	①	
Television	0	1	
Radio	0	1	Total _____
Refrigerator	0	1	
Bicycle	0	1	
Motorcycle	0	1	
Car	0	1	

C. STUDENT'S HEALTH BACKGROUND

15. Where was _____ born?

Home	_____	(0)
Hospital	_____	(1)
Other	_____	(9)

16. How much did s/he weight?

_____ lbs   _____ oz

17. if not known, was s/he

small	_____	(0)
okay	_____	(1)
big	_____	(2)
unknown	_____	(9)

18. Has _____ been hospitalized?

chest infect.	_____	(0)
diarrhoea	_____	(1)
Other	_____	(9)
No	_____	(2)

19. Does _____ have any chronic disease? (i.e. asthma)

Yes	_____	(0)
No	_____	(1)

D. SCHOOL AND STIMULATION

20. How many years has student been at primary school? _____

    How old, in months, was student when entered school? _____

21. How often does your child have to miss school?		22. How often does your child miss school because there is not enough money?	
Never goes to school	0	more than half the days/month	0
Often misses 4 days per week	1	7-15 days per month	1
Often misses 2/3 days per week	2	3-6 days per month	2
Often misses one day per week	3	1-2 days per month	3
Only when sick	(4)	Never misses	(4)
23. Do you or another adult at home ever teach school work in the past year? who?		24. Have you or any other adult had time to read any other book with your child in the past year? Who?	
Never	0	Never	(0)
Once per month	1	Once per month	1
Once per week	(2)	Once per week	2
More than once per week	3	More than once per week	3
Every day	4	Every day	4
25. Have you or another adult at home ever told the child stories in the past year? Who?		26. How often has the child watched television, in the past year?	
Never	0	Never	0
Once per month	1	Once per month	1
Once per week	(2)	Once per week	(2)
More than once per week	3	More than once per week	3
Every day	4	Every day	4
27. Have you or another adult at home played with toys, puzzles, other games in the past year? Who?		28. Have you taken the child on trips or outings in the past year? (i.e. gardens, exhibitions) Who?	
Never	0	Never	0
Once per month	(1)	One outing	(1)
Once per week	2	2-3 outings	2
More than once per week	3	4-10 outings	3
Every day	4	At least once per month	4

29. How many of each of the following does the child have:

exercise books	_____
text books	_____
fun books	_____
other books	_____
pencils/pens	_____
crayons/markers	_____
slate/papers	_____
toys	_____
(please list)	

30. Does the child have a table/chair at home to use for homework? (Y=1, N=0)

table	_____
chair	_____

```
(1)              /aggression
(1 1)            /aggression/personal
(1 1 1)          /aggression/personal/punishment
(1 1 1 1)        /aggression/personal/punishment/corporal
(1 1 1 1 1)      /aggression/personal/punishment/corporal/teacher
(1 1 1 1 2)      /aggression/personal/punishment/corporal/parent
(1 1 1 2)        /aggression/personal/punishment/non-corporal
(1 1 1 2 1)      /aggression/personal/punishment/non-corporal/teacher
(1 1 1 2 2)      /aggression/personal/punishment/non-corporal/parent
(1 1 2)          /aggression/personal/fight
(1 1 2 1)        /aggression/personal/fight /bully
(1 1 2 2)        /aggression/personal/fight /victim
(1 2)            /aggression/community
(1 2 1)          /aggression/community/killing
(2)              /parents
(2 1)            /parents/father
(2 1 1)          /parents/father/work outside home
(2 1 2)          /parents/father/housework
(2 1 3)          /parents/father/financial support
(2 1 4)          /parents/father/childcare
(2 1 5)          /parents/father/fun things
(2 1 6)          /parents/father/education
(2 1 7)          /parents/father/love
(2 1 8)          /parents/father/independent activity
(2 1 9)          /parents/father/violence, punishment
(2 1 10)         /parents/father/miscellaneous
(2 1 11)         /parents/father/work at home
(2 1 12)`        /parents/father/outside home and money.
(2 1 13)         /parents/father/fun and love
(2 1 14)         /parents/father/misc + indep
(2 2)            /parents/mother
(2 2 1)          /parents/mother/work outside home
(2 2 2)          /parents/mother/housework
(2 2 3)          /parents/mother/financial support
(2 2 4)          /parents/mother/childcare
(2 2 5)          /parents/mother/fun things
(2 2 6)          /parents/mother/education
(2 2 7)          /parents/mother/love
(2 2 8)          /parents/mother/independent activity
(2 2 9)          /parents/mother/violence, punishment
(2 2 10)         /parents/mother/miscellaneous
(2 2 11)         /parents/mother/work at home
(2 2 12)         /parents/mother/outside home and money
(2 2 13)         /parents/mother/fun and love
(2 2 14)         /parents/mother/misc + independent
(2 3)            /parents/linked together
(3)              /school
(3 1)            /school/what happens
(3 1 1)          /school/what happens/violence
(3 1 2)          /school/what happens/play, sports, fun
(3 1 3)          /school/what happens/learning related
(3 1 4)          /school/what happens/routines
(3 1 5)          /school/what happens/negative, punishment, unpleasant
(3 1 6)          /school/what happens/miscellaneous
(3 1 7)          /school/what happens/violence or punishment
(3 2)            /school/teacher
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(3 2 1)              /school/teacher/violence
(3 2 2)              /school/teacher/play, sports, fun
(3 2 3)              /school/teacher/learning related
(3 2 4)              /school/teacher/routines
(3 2 5)              /school/teacher/negative
(3 2 6)              /school/teacher/miscellaneous
(3 2 7)              /school/teacher/violence and punishment
(3 3)                /school/principal
(3 3 1)              /school/principal/violence
(3 3 2)              /school/principal/play, sports, fun
(3 3 3)              /school/principal/learning related
(3 3 4)              /school/principal/routines
(3 3 5)              /school/principal/negative, punishment, unpleasant
(3 3 6)              /school/principal/miscellaneous
(3 3 7)              /school/principal/violence and punishment
(4)                  /problem situation
(4 1)                /problem situation/passive situation assessment
(4 2)                /problem situation/immediate response
(4 2 1)              /problem situation/immediate response/emotional
(4 2 2)              /problem situation/immediate response/physiological
(4 2 3)              /problem situation/immediate response/physical action
(4 3)                /problem situation/active problem solving
(4 3 1)              /problem situation/active problem solving/negative
(4 3 2)              /problem situation/active problem solving/positive
(4 5)                /problem situation/tell "grown-up"
(4 6)                /problem situation/punishment
(4 6 1)              /problem situation/punishment/corporal
(4 6 2)              /problem situation/punishment/non-corporal
(4 7)                /problem situation/interactions or reactions
(4 7 1)              /problem situation/interactions or reactions/friends
(4 7 2)              /problem situation/interactions or reactions/parents
(4 7 3)              /problem situation/interactions or reactions/teachers
(4 8)                /problem situation/miscellaneous
(5)                  /policeman
(5 1)                /policeman/protective
(5 2)                /policeman/negative
(5 3)                /policeman/routines
(5 4)                /policeman/punishment
(5 5)                /policeman/violence
(5 6)                /policeman/miscellaneous
(D)                  //Document Annotations
(F)                  //Free Nodes
(T)                  //Text Searches
(I)                  //Index Searches
(C)                  //Node Clipboard - 'physical action'
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