Is relative leg length a biomarker of childhood nutrition? Long-term follow-up of the Hyderabad Nutrition Trial

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Background Relative leg length is frequently used as a biomarker of childhood nutrition in epidemiological studies, but evidence is lacking. We examined the association between supplemental nutrition in pregnancy and childhood and relative proportions of components of height in adolescence.

Methods In a community trial of nutritional supplementation, villages from adjacent administrative areas were selected to serve as intervention (n = 15) and control (n = 14) arms. In the intervention villages, balanced protein–calorie supplementation (2.51 MJ, 20 g protein) was offered daily to pregnant women and their offspring until the age of 6 years. Children born in the trial were re-examined 15 years later to assess components of height.

Results A total of 1165 adolescents (intervention: 654, 49% of trial participants; control: 511, 41% of trial participants) aged 13–18 years were examined. Supplemented children were 10 mm taller [95% confidence interval (CI): 1.4 to 18.7 mm], but almost all of the increase was in trunk length (9 mm, 95% CI: 2.6 to 15.4 mm). The age- and gender-adjusted β-coefficients for the association of nutritional supplementation with relative trunk, leg and lower leg lengths (expressed as standard deviation scores) were 0.26 (95% CI: 0.11 to 0.42), 0.08 (95% CI: −0.03 to 0.19) and 0.03 (95% CI: −0.08 to 0.15) respectively, thereby unsupportive of cephalocaudal gradient in growth.

Conclusions In this nutritional supplementation trial in an undernourished population, we were unable to confirm relative leg length as a biomarker of childhood nutrition. Alternative explanations may underlie the reported associations between childhood conditions and relative leg length.

Keywords Leg length, childhood nutrition, risk factors, cohort studies, community trial
Introduction
Relative leg length (leg length as a proportion of stature) is increasingly used as a biomarker of childhood nutrition in epidemiological studies. The theoretical basis for its use was provided >50 years ago by Isabel Leitch, who used animal models and the cephalocaudal gradient in mammalian growth to argue that ‘children continuously underfed would grow into underdeveloped adults…with normal or nearly normal size head, retarded trunk and relatively short legs’ (p. 145). Others using the same line of argument (i.e. cephalocaudal gradient) have suggested that relative lower leg length may be even more sensitive as a biomarker of childhood nutrition than leg length. However, despite its obvious importance as a tool for epidemiological research and evaluation of nutrition policy, evidence that the cephalocaudal gradient in human growth is modifiable by nutritional influences in childhood remains circumstantial or weak.

At a population level, increased leg length has contributed substantially to secular trends in height increases in Japan, Europe and the USA. In cohort studies from some Western countries, relatively shorter leg length has been associated with poorer socio-economic conditions in childhood, inadequate nutrition is suggested as an explanation, although recent data from non-Western countries, where undernutrition is more of a problem, are inconsistent. In a non-randomized trial conducted in 1930’s Britain, food supplements given to children aged 2–14 years were associated with a small relative increase in leg length over the 1-year supplementation period. In a British birth cohort, energy intake at 4 years was modestly associated with leg length (but not trunk length) at the age of 43 years. In two other British cohorts, however, maternal diet in pregnancy or dietary intake in childhood were not associated with leg length in childhood. The lack of evidence is attributable largely to absence of suitable cohort studies. Furthermore, since many of the childhood exposures including nutrition are socially patterned, trials remain the only reliable means of establishing causality. In 29 villages near Hyderabad city in India, we re-examined children born in a community trial of nutritional supplementation of pregnant women and young children after 15 years, and carefully assessed the components of their height. We hypothesized that: (i) relative leg length would be more strongly associated with nutritional supplementation than relative trunk length; and (ii) relative lower leg length would be more strongly associated with nutritional supplementation than relative leg length.

Methods
The details of the trial and the follow-up have been published. In brief, a controlled trial was conducted in 29 villages near Hyderabad city in South India (1987–90), using the opportunity afforded by the stepwise expansion of a nutritional supplementation programme (the Integrated Child Development Services scheme). In two adjacent administrative areas (one with ongoing programme, the other awaiting implementation), all villages within a 20 km radius of a central village were selected to serve as intervention \((n = 15)\) and control \((n = 14)\) areas, with unselected intervening villages to avoid contamination. In the intervention villages only, a nutritional supplement (made of corn–soya blend and soybean oil) was available daily to all pregnant and lactating women and children <6 years of age, providing on average 2.09 MJ and 20–25 g protein to pregnant/lactating women and 1.25 MJ and 8–10 g protein to children. No other nutrients (e.g. micronutrients) were added to the supplement, and other national programmes (e.g. immunization, anaemia control and basic healthcare) were available in both the control and intervention areas.

Measurements
Children born during the trial were traced in 2002–05 and invited to undergo examination at clinics conducted in the villages. An interviewer-administered questionnaire was used to collect socio-demographic, health and lifestyle information. Socio-economic position was assessed by the Standard of Living Index (SLI), which is a household level, asset-based scale devised for use in India. The SLI has 29 items (e.g. quality of housing, toilet facilities, source of lighting and drinking water, land and animal ownership and possession of material goods such as radio, television, bicycle, car, tractor, refrigerator, etc.), and is particularly suitable for use in rural India, where the joint family structure and subsistence economy render an individual’s own income a problematic measure. We additionally collected data on village population from the village heads, as an index of village urbanization.

Height was measured with a portable stadiometer (Leicester height measure; Chasmors Ltd, London, UK). The participant stood erect with the head in the Frankfort plane, and a gentle upward pressure applied under the mastoid. Sitting height was measured with the subject sitting on a stool of known height, and legs hanging unsupported over the edge. With the participant still sitting on the stool and the left ankle or calf placed on the right knee, lower leg length (below knee) was measured with a Chasmors calipers (Chasmors Ltd, London, UK), by applying the caliper blades from the proximal–medial border of the tibia to the distal border of the medial malleolus. Weight was measured with a digital weighing machine (HD 305; Tanita, Tokyo, Japan). Skinfold thickness was measured in triplicate at four sites (biceps, triceps, subscapular and suprailiac) with a Holtain caliper (supplied by Chasmors Ltd, London, UK). Sexual maturation was
classified into four stages (corresponding to Tanner’s early, middle, late and post-puberty) on the basis of time since the onset of menstruation (girls) and tes-
ticular volume (boys). 26 Testicular volume was self-
assessed by children in private, using Prader’s orchid-
ometer (chain of 12 wooden testes, volumes ranging
from 1 to 25 ml).

Quality control
Standardization of the data collection was achieved
through detailed protocols and regular training ses-
sions and anthropometric equipment was calibrated
daily. Only one observer was used for each measure-
ment to eliminate inter-observer bias. Reproducibil-
ity of clinic measurements was assessed by repeating
the measurements on a random sub-sample (5%) of par-
ticipants after 1–3 weeks, and was found to be con-
sistently high, with intra-class correlation coefficients
of >0.98 for all anthropometric measures. Testicular
self-assessment technique was validated against a
trained observer in a separate sub-study conducted
in a local school and found to be highly accurate:
mean difference in model ranks (self-reported minus
directly observed) was 0.07 [95% confidence interval
(CI): −0.11 to 0.25], with no evidence of systematic
bias on Bland–Altman plot. 27

Statistical analyses
Trunk length was estimated as the difference between
sitting height and stool height. Leg length was esti-
more the difference between standing height and
and trunk length. The relative components of height
were derived as follows: relative trunk length = (trunk
length/height)*100; relative leg length = (leg length/
height)*100; and relative lower leg length = (lower
leg length/height)*100. To allow direct comparisons
between the effect estimates of anthropometric mea-
ures, height and its relative components were con-
verted to internally derived standard deviation scores
[SDS = (observed value – mean value)/SD], using the
age- and sex-specific means and SDs of the study
population. The log of the sum of four skinfolds
was used to calculate the percentage of body fat, 28
which was converted to fat and fat-free mass using
bodyweight, and expressed as relevant indices by
dividing with the square of height in metres. 29
Central adiposity was assessed by the ratio of central
(sub-scapular plus suprailiac) to peripheral (biceps
plus triceps) skinfolds. The SLI was used to classify
the participants as low (0–14), medium (15–24) or
high (25–67) socio-economic position, as recom-

Ethics
Ethical approval for the study was obtained from the
ethics committee of the National Institute of
Nutrition, Hyderabad. Approval was also sought
from the village heads and their committees in each
of the 29 villages. Written informed consent (wit-
nessed thumbprint if illiterate) was obtained from
the participants and their parents (or guardians).
Results

At the follow-up assessment, 1963 (71%) families could be traced successfully to the original trial. Of the 2601 children eligible for study inclusion (i.e. born to these families during 1987–90 and still alive in 2002–05), only those with baseline information from the trial \( (n = 1492; 57\%) \) were invited for clinical examination. A total of 1165 children participated in the clinics: 654 (82%) in the intervention and 511 (74%) in the control area, representing 45% (49% intervention, 41% control) of all births during the trial period (for flow diagram of recruitment, see earlier publication).\(^2\) Children who took part in the clinics were similar to those who were eligible but did not participate.\(^2\) After excluding 3 pregnant girls, there were 1162 participants. Complete data on anthropometric measures and relevant confounders were available for 1120 (96%) adolescents. The average number of participants from each village was 40 (range: 2–122).

The median age of the participants at the follow-up assessment was 15.7 years (range: 13–18 years), of which 54% were boys. The socio-demographic characteristics and pubertal stage of the participants were broadly similar across the intervention and control areas (Table 1). Only two children reported tobacco use or alcohol consumption, so these variables were not considered in the analyses. The relatively short stature (boys: 158.8 cm; girls: 151.5 cm) and low body mass index (boys: 16.7 kg/m\(^2\); girls: 17.8 kg/m\(^2\)) of the participants is consistent with a chronically undernourished population. Participants in the intervention area were relatively taller (mean difference 10 mm, 95% CI: 1.4 to 18.7 mm), but had broadly similar body composition to those in the control areas, apart from a slightly greater fat-free mass index in controls. In unadjusted comparisons,

| Table 1 | Participant characteristics by area of intervention at follow-up in the Hyderabad Nutrition Trial |
|---|---|---|---|---|
| | **n** | **Intervention** | **Control** | **Mean difference (95% CI)** |
| **Socio-demographics** | | | | **P-value** |
| Age (years) | 1162 | 15.8 (0.9) | 15.9 (0.9) | 0.12 (−0.41 to 0.17) |
| Male, n (%) | 1162 | 345 (53) | 282 (55) | 0.4 |
| Pubertal stage, n (%) | 1124 | | | 0.4 |
| Early puberty | 88 (14) | 83 (17) | | |
| Middle puberty | 209 (33) | 156 (31) | | |
| Late puberty | 184 (29) | 132 (27) | | |
| Post-puberty | 147 (23) | 125 (25) | | |
| Standard of Living Index, n (%) | 1128 | | 0.2 |
| High (25–67) | 228 (36) | 158 (32) | | |
| Medium (15–24) | 291 (46) | 239 (48) | | |
| Low (0–14) | 111 (18) | 101 (20) | | |
| **Anthropometry** | | | | |
| Height (mm) | 1120 | 1559 (83) | 1549 (82) | 10.04 (1.40 to 18.69) |
| Trunk length (mm) | 1120 | 804 (47) | 794 (47) | 9.01 (2.62 to 15.40) |
| Leg length (mm) | 1120 | 755 (47) | 754 (46) | 1.03 (−4.20 to 6.27) |
| Lower leg length (mm) | 1120 | 376 (24) | 376 (23) | 0.25 (−3.14 to 2.64) |
| Relative trunk length\(^a\) (%) | 1120 | 51.6 (1.4) | 51.3 (1.4) | 0.25 (0.001 to 0.5) |
| Relative leg length\(^a\) (%) | 1120 | 48.4 (1.4) | 48.7 (1.4) | 0.25 (−0.5 to −0.001) |
| Relative lower leg length\(^a\) (%) | 1120 | 24.1 (0.7) | 24.3 (0.7) | −0.18 (−0.31 to −0.04) |
| Fat mass index (kg/m\(^2\)) | 1120 | 2.6 (1.3) | 2.6 (1.5) | 0.02 (−0.19 to 0.23) |
| Fat-free mass index (kg/m\(^2\)) | 1120 | 14.5 (1.4) | 14.8 (1.6) | −0.29 (−0.57 to −0.01) |
| Central–peripheral skinfold ratio | 1120 | 1.48 (0.25) | 1.47 (0.25) | 0.01 (−0.05 to 0.06) |

Intervention and control values are means (SD) unless stated otherwise. Differences for continuous variables are based on linear regression models with robust standard errors to account for clustering by village. \( P \)-values for categorical variables are based on unpaired \( t \)-tests or \( \chi^2 \) tests for heterogeneity with appropriate degrees of freedom.

\( ^a \)Relative lengths were estimated as a proportion of height, i.e. relative trunk length = (trunk length/height)\(^\times\)100; relative leg length = (leg length/height)\(^\times\)100; relative lower leg = (lower leg length/height)\(^\times\)100.
most of the difference in height arose from a difference in trunk length (mean difference 9 mm, 95% CI: 2.6 to 15.4 mm), with little difference in leg length or lower leg length of the intervention and control participants. A greater proportion of height was composed of trunk length in the intervention area, compared with control.

Adjusting for age and gender, children in the intervention areas were on average 0.17 SDS (95% CI: 0.08 to 0.27) taller than children in the control areas (Table 2). Relative trunk length was the component of height most strongly associated with nutritional supplementation (SDS: 0.26, 95% CI: 0.11 to 0.42), relative leg length was intermediate (SDS: 0.08, 95% CI: 0.03 to 0.19) and relative lower leg length was the least (SDS: 0.03, 95% CI: 0.08 to 0.15), arguing against a cephalocaudal gradient in growth. The associations of nutritional supplementation with components of height were largely unaltered by adjustments for pubertal stage, socio-economic position, sibling number (and birth order) or body composition of the participants. Adjustment for body composition inflated the variance somewhat with little change in the beta itself; this may have resulted either because these additional variables contributed little to the effect estimate or from potentially greater degree of measurement error in body composition variables that were secondarily derived from skinfolds using formulae. The $R^2$ statistic in these multivariable models ranged from 0.002 to 0.19, suggesting that a considerable proportion of the variation in components of height in this cohort was explained by factors other than nutritional supplementation. Although there were some differences in the effect estimates between the sexes (possibly as a result of differences in maturational stages and wider confidence intervals in smaller sample sizes), they did not change the conclusions in any material way (Supplementary Table S1, Supplementary data are available at IJE online).

Discussion
In this long-term follow-up of a trial conducted in a chronically undernourished population, balanced protein–calorie supplementation given to pregnant women and young children was associated with a moderate increase in offspring height. Almost all of the difference in height was in the trunk area, with little change in the relative proportions of leg or lower leg lengths. The absence of a disproportion in lower limbs argues against a strong increase in lower limits of the cephalocaudal gradient in human sensitivity to nutritional influences in pregnancy and early childhood. These data do not support the use of relative leg length as a biomarker of childhood nutrition.

Table 2 Association of nutritional supplementation with components of height in the Hyderabad Nutrition Trial

<table>
<thead>
<tr>
<th>Component</th>
<th>Model 1</th>
<th>P-value</th>
<th>Model 2</th>
<th>P-value</th>
<th>Model 3</th>
<th>P-value</th>
<th>Model 4</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Height SDS</td>
<td>0.20 (0.09 to 0.31)</td>
<td>0.001</td>
<td>0.20 (0.07 to 0.33)</td>
<td>0.004</td>
<td>0.18 (0.04 to 0.32)</td>
<td>0.012</td>
<td>0.17 (−0.02 to 0.35)</td>
<td>0.071</td>
</tr>
<tr>
<td>Relative trunk length SDS</td>
<td>0.26 (0.11 to 0.42)</td>
<td>0.001</td>
<td>0.26 (0.09 to 0.43)</td>
<td>0.004</td>
<td>0.23 (0.06 to 0.41)</td>
<td>0.011</td>
<td>0.24 (0.02 to 0.45)</td>
<td>0.032</td>
</tr>
<tr>
<td>Relative leg length SDS</td>
<td>0.08 (−0.03 to 0.19)</td>
<td>0.170</td>
<td>0.08 (−0.04 to 0.19)</td>
<td>0.172</td>
<td>0.07 (−0.05 to 0.19)</td>
<td>0.243</td>
<td>0.04 (−0.10 to 0.18)</td>
<td>0.574</td>
</tr>
<tr>
<td>Relative lower leg length SDS</td>
<td>0.03 (−0.08 to 0.15)</td>
<td>0.545</td>
<td>0.03 (−0.09 to 0.15)</td>
<td>0.576</td>
<td>0.03 (−0.10 to 0.15)</td>
<td>0.658</td>
<td>0.01 (−0.13 to 0.15)</td>
<td>0.875</td>
</tr>
</tbody>
</table>

Model 1 adjusted for age and gender; Model 2 adjusted for variables in Model 1 plus pubertal stage; Model 3 adjusted for variables in Model 2 plus socio-economic circumstances (household Standard of Living Index and village population) plus supplement availability within households (number of siblings plus birth order); Model 4 adjusted for variables in Model 3 plus body composition (fat mass index, fat–free mass index, central–peripheral skinfold ratio). $n = 1120$ for Models 1 and 2 and $n = 1068$ for Models 3 and 4.

*Relative lengths were estimated as a proportion of height [i.e. relative trunk length = (trunk length/height)$^{100}$; relative leg length = (leg length/height)$^{100}$; relative lower leg = (lower leg length/height)$^{100}$] and subsequently converted into SDS, which were age–sex standardized to the study population. Results are based on linear regression models with robust standard errors to account for clustering by village; β (95% CI) are for differences between the two areas (intervention minus control).
Comparison with previous research and mechanisms

Despite convincing animal models, there is little evidence to suggest that the cephalocaudal gradient in growth is modifiable in humans by nutritional manipulation.\textsuperscript{10} Few studies have examined the relationship between childhood nutrition and relative leg length. In a non-randomized trial conducted in 1930's Britain, food supplements given to children aged 2–14 years over a 1-year period were associated with a 3.7-mm increase in height relative to the controls.\textsuperscript{19} Most of the increase in height came from increases in leg length (3.3 mm), precisely cristal height (distance from the floor to the summit of the iliac crest measured with a steel tape). However, the authors advised caution in interpreting this finding due to difficulties in measuring cristal height accurately; in sub-group analyses, leg length increases were greater than those for overall height, which is implausible. In a follow-up of the 1946 British births, energy intake at the age of 4 years (assessed by 24-h diet recall from the mother) was modestly associated with leg length at 43 years, but not trunk length.\textsuperscript{8} Conversely, in two other British cohorts, maternal diet in pregnancy or dietary intake in childhood was not associated with leg length at ages 7.5 and 2–14 years respectively.\textsuperscript{16,20} As far as we are aware, no study has examined the association of childhood nutrition with lower leg length.

How can these findings be reconciled with disproportionately greater contribution of leg length to secular increases in height,\textsuperscript{11,13,14} and leg length's independent association with childhood socio-economic conditions in some Western countries?\textsuperscript{15,16} The few data on the association of childhood nutrition with relative leg length do not equate to evidence against an association and may simply reflect an absence of appropriate cohort or experimental studies. Alternatively, the nutritional influences on growth could be trans-generational (mediated over multiple generations): there is evidence to suggest that improvements in nutrition are reflected in the growth of subsequent generation.\textsuperscript{14} Such effects may arise as a result of a physical need for improvement in the size of the mother preconceptionally, or mediated through nutritionally driven epigenetic influences that may constrain growth in recently developed populations to avoid the biological costs of too rapid catch-up.\textsuperscript{14,30} In long-term undernourished populations, recent improvements in nutrition may in the first instance be directed preferentially to important areas such as brain or vital organ systems (at the cost of physical size),\textsuperscript{31} and this was evidenced by important reductions in cardiovascular risk associated with nutritional supplementation in this cohort.\textsuperscript{22} The modest effects of intra-generational nutritional influences on height (0–22 mm in trials)\textsuperscript{19,32,33} suggest that substantial variations in background socio-economic conditions (at critical periods in childhood) would be necessary to detect their associations with relative components of height. This may explain why strong socio-economic differentials in relative leg length are reported in some older cohorts from Western countries,\textsuperscript{15,16} but not those from non-Western countries (which may have had uniformly deprived conditions).\textsuperscript{17,18}

Limitations

The main limitation of this study is that the villages were not formally randomized. The 100 and more villages in each of the two administrative areas were spread over an unfeasibly large geographical area. However, all villages within a radius of a central village in the intervention and control areas were chosen, making any important selection bias unlikely. The exclusion of a large number of intervening villages additionally limited the potential for cross-over of the participants between intervention and control areas. A second limitation is the substantial loss to follow-up. A higher proportion of participants in the controls areas could not be traced because they had relatively more temporary residents at the time of the trial. This was compounded by reduced clinic participation in the follow-up survey in some of the control villages due to concurrent political elections. Loss to follow-up resulting from these reasons is not likely to be systematic, as supported by a lack of notable differences in the baseline characteristics of participants and non-participants.\textsuperscript{22}

The nutritional supplement was offered not only to children, but also to pregnant and lactating women. The effect of diet in pregnancy or infancy on leg length or other components of height is unknown.\textsuperscript{20} The intake of the supplement was not directly observed; furthermore, it is recognized that only about half of the participants collect supplements in such programmes, which is often shared between family members.\textsuperscript{34} Adjustment of the models for sibling number and birth order (as a proxy for differential availability of supplement within households) made no material difference to the results. Increased uptake of other components of the programme such as immunization may also have had an impact on nutrition. These concerns introduce considerable uncertainty to the conclusions. Although difficult to predict with confidence, the likely effect of these potential biases would be to underestimate the true effect of the intervention. Not all participants had completed their growth at the time of assessment; although the results may change in adulthood, the predominantly pre-pubertal timing of lower limb growth (as opposed to trunk which grows more during puberty) suggests that the conclusions are likely to be strengthened, if anything, with further growth in puberty.\textsuperscript{35}

The primary outcome of this study was a change in relative proportions of height's components. This may have benefited the study in two unintended ways. First, unlike an absolute measure such as height, a relative measure (such as leg:height ratio) may be
partially robust to a moderate bias in sample selection, since all of the components may be similarly subject to the same selection bias, rendering it unimportant. Secondly, the fieldworkers may have held a prior belief on the effects of supplemental nutrition on height, but less so on its effects on relative components of height. Since ‘blinding’ of the fieldworkers to intervention was not an option, this may have provided some protection from observer bias.

Implications
The current level of evidence does not justify the routine use of relative leg length as a biomarker of childhood nutrition in epidemiological studies or as a policy tool to assess the impact of nutritional programmes. More research is needed to understand the mechanisms underlying the strong differentials in relative leg length associated with childhood socio-economic conditions in some western countries.

Supplementary Data
Supplementary data are available at IJE online.

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Conflict of interest: None declared.

KEY MESSAGES
- In this long-term follow-up of a trial conducted in a chronically undernourished population, balanced protein–calorie supplementation given to pregnant women and young children was associated with a moderate increase (10 mm) in offspring height.
- Almost all of the increase in height was in the trunk region (9 mm) with hardly any change in total leg length (1 mm) or below knee leg length (0 mm).
- These data suggest that leg length may not be as reliable a biomarker of childhood undernutrition as previously believed; consequently more data are needed before it is universally adopted for this use.
- Since the trial was conducted within the framework of a multi-component public health programme, the absolute magnitude of changes in height components may not be completely reliable; however, the changes in height components, relative to each other, are likely to be reasonable.

References


