Title: Constraint and Tradeoffs Regulate Energy Expenditure During Childhood

SHORT TITLE: Constraint and Tradeoffs in Child Energetics

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ABSTRACT
Children's metabolic energy expenditure is central to evolutionary and epidemiological frameworks for understanding variation in human phenotype and health. Nonetheless, the impact of a physically active lifestyle and heavy burden of infectious disease on child metabolism remains unclear. Using energetic, activity, and biomarker measures, we show that Shuar forager-horticulturalist children of Amazonian Ecuador are ~25% more physically active and, in association with immune activity, have ~20% greater resting energy expenditure than children from industrial populations. Despite these differences, Shuar children's total daily energy expenditure, measured using doubly labeled water, is indistinguishable from industrialized counterparts. Tradeoffs in energy allocation between competing physiological tasks, within a constrained energy budget, appear to shape childhood phenotypic variation (e.g., patterns of growth). These tradeoffs may contribute to the lifetime obesity and metabolic health disparities that emerge during rapid economic development.

ONE-SENTENCE SUMMARY
Forager-horticulturalist children do not spend more calories than industrialized children, but they do spend calories differently.

INTRODUCTION
Metabolic energy is needed to perform life's essential tasks, including somatic maintenance (e.g., immune activity, cellular repair), growth, reproduction, and physical

activity. Life history theory proposes that organisms have evolved to adaptively manage the allocation of energetic resources between these competing tasks, resulting in tradeoffs that shape phenotypic variation across the life course (*1, 2*). Energetic tradeoffs and evolved constraints on overall total energy expenditure (TEE; kcal/d) have been demonstrated for numerous species (*3-6*), including humans (*7-11*).

Despite wide acceptance of energetic tradeoffs and constraint in evolutionary biology, prevailing models in human health and nutrition assume that energy use is additive (*12*). Such additive TEE models suggest that, rather than engendering tradeoffs, caloric investment in any single metabolic task correspondingly increases TEE in an unbounded, dose-dependent manner (Fig. 1A). The Additive TEE Model implies large differences in energy budgets between populations owing to differences in lifestyle and environment. Among industrialized populations, increasingly sedentary lifestyles and minimal pathogen burdens are assumed to lower TEE, thereby promoting positive energy imbalance and accelerating the global pandemic of obesity and metabolic disease that accompanies secular dietary change (*13*).

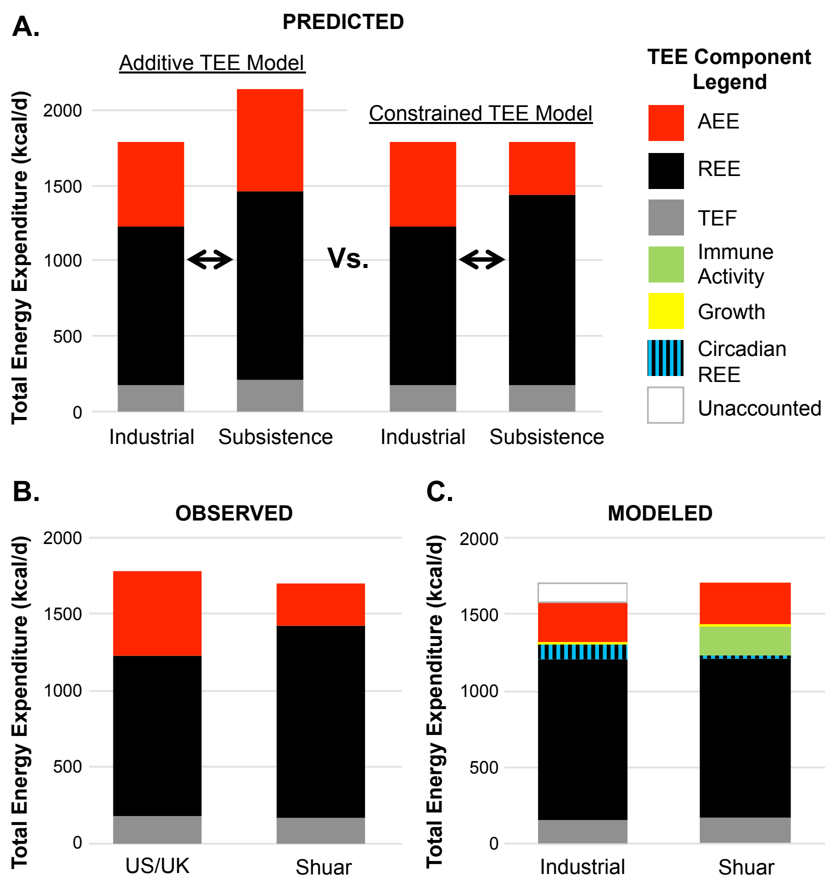

Fig. 1. Predicted Additive TEE and Constrained TEE Models of human energy use (A), with observed (B) and modeled (C) components of TEE among Shuar and industrialized children. A. Children from subsistence-based populations with relatively heavy burdens of infectious disease and active lifestyles are predicted to either increase TEE as a result of increased REE and AEE (Additive TEE Model) or maintain TEE at industrialized levels as a result of REE and AEE tradeoffs (Constrained TEE Model). **B.** Observed energy expenditures in Shuar and US/UK cohorts follow the

Constrained TEE Model, with greater Shuar REE but lower AEE and no overall TEE difference. **C.** Modeled energy expenditures accounting for immune activity and growth as well as possible population differences in exercise efficiency and REE circadian variation produce TEE estimates for Shuar and Industrial populations that vary by only 8% (*Materials and Methods*). TEE = total energy expenditure; REE = resting energy expenditure; AEE = activity energy expenditure; TEF = thermic effect of food.

Determining whether human energy expenditure is constrained or additive is critical for understanding and reversing global trends in obesity and poor metabolic health. The implications of this understanding are perhaps most salient during childhood, a uniquely human life stage (*14*) that is exceptionally energetically demanding (*9*) and is critical in establishing lifetime trajectories of phenotype and metabolic health (*15*). Problematically, no studies of childhood TEE regulation to date have been performed among cohorts with chronically high levels of immune and physical activity. Nor have they included the objective measures of resting energy expenditure (REE; kcal/d) and daily physical activity necessary to identify possible energy allocation tradeoffs.

Here, we test for constraint and tradeoffs in childhood energy expenditure using doubly labeled water (DLW) measures of TEE and respirometry measures of REE from forager-horticulturalist Shuar in Amazonian Ecuador. The Shuar engage in physically active, subsistence-based lifestyles and experience persistent immune activation (*7*). We compared Shuar TEE and REE, as well as activity energy expenditure (AEE; the portion of TEE not attributed to REE or digestion), physical activity level (PAL; TEE/REE), and measures of daily physical activity (from accelerometry) to data from children living in industrialized populations (*16-18*). The Additive TEE Model predicts that Shuar TEE should be elevated relative to industrial references, reflecting greater investment in immune activity (increased REE) and physical activity (increased AEE). In contrast, the Constrained TEE Model predicts that TEE should be similar between populations, the result of tradeoffs between underlying energy expenditure components.

RESULTS

High Shuar resting energy expenditure and daily physical activity

Shuar REE and daily physical activity were elevated compared to industrialized children. In multivariable analysis controlling for age, sex, log-FM, and log-FFM, Shuar REE exceeded that of US/UK children by 213 kcal/d or 20% (Fig. 2, Table 1, table S1; $\beta = 0.19$, $SE = 0.03$, $p < 0.001$). Likewise, Shuar accelerometry averaged 96 counts/min (25%) more than Canadian references (Fig. 2, Table 1; two-sample $t[2883] = 3.86$, $p < 0.001$), with Shuar spending 31 min/d (53%) more time than industrialized children in moderate-vigorous physical activity (MVPA; Fig. 2, Table 1; two-sample $t[2883] = 6.08$, $p < 0.001$).

Table 1. Measures of interest for Shuar and industrialized cohorts.

	Population	
	Shuar (N = 44)	Industrial (N = 40)[†]
Descriptive (mean [95% CI])		
Sex (% male)	50%	56%
Age (yrs)	8.1 (7.5 – 8.7)*	7.1 (6.7 – 7.5)
Anthropometry (adjusted mean [95% CI])		
Stature (cm)	117.0 (115.2 – 118.8)***	124.6 (122.7 – 126.4)
Body mass (kg)	22.8 (21.6 – 24.0)***	26.0 (24.7 – 27.3)
Body mass index (kg/m^2)	16.5 (16.1 – 17.0)	16.5 (16.1 – 17.0)
Fat mass (kg)[a]	2.7 (2.4 – 3.0)***	5.7 (5.0 – 6.4)
Fat-free mass (kg)[a]	19.7 (18.9 – 20.3)	19.5 (18.7 – 20.3)
Body fat percentage (%)[a]	11.9% (11.0 – 12.9)***	22.4% (20.5 – 24.5)
Energetics (adjusted mean [95% CI])		
Total energy expenditure (kcal/d)[a]	1738 (1670 – 1809)	1811 (1733 – 1892)
Resting energy expenditure (kcal/d)[a]	1255 (1215 – 1296)***	1042 (1006 – 1080)
Activity energy expenditure (kcal/d)[a]	276 (225 – 338)***	558 (447 – 698)
Physical activity level	1.38 (1.31 – 1.46)***	1.76 (1.68 – 1.84)
	Shuar (N = 30)	Industrial (N = 2855)[‡]
Daily Physical Activity (mean [95% CI])		
Activity wear time (hrs/d)	12.2 (11.6 – 12.8)***	13.5 (13.4 – 13.6)
Activity counts (counts/min)	474 (433 – 516)***	379 (365 – 392)
Sedentary activity (min/d)	240 (228 – 252)***	478 (473 – 483)
Light activity (min/d)	405 (382 – 428)***	273 (268 – 278)
Moderate-vigorous activity (min/d)	89 (76 – 102)***	58 (55 – 61)[§]

[†]US/UK cohort (*16, 17*); [‡]Canadian cohort (*18*); [§]UK sample reported 64 min/d of moderate-vigorous activity (*17*); [a]Values back-converted from analysis of log-transformed measures (table S1); *p < 0.05; ***p < 0.001; Population-level differences in two-tailed t-tests (Daily Physical Activity measures) or multivariable models controlling for age and sex (Anthropometry measures) or age, sex, log-FM, and log-FFM (Energetics measures).

No difference in Shuar total energy expenditure

Despite greater REE and daily physical activity, Shuar TEE did not differ from that of children in industrialized populations. Controlling for age, sex, log-FM, and log-FFM, there was no difference between Shuar and US/UK TEE values (Fig. 2, Table 1, table S1; $\beta = -0.04$, $SE = 0.04$, $p = 0.258$; alternative models give similar results, table S2). Results were consistent in analysis of mean TEE values from a larger and more diverse sample of children in industrialized countries (fig. S1). These results support the Constrained TEE Model of energy use.

Low Shuar activity energy expenditure and physical activity level

Shuar children spent 72% of their total energy budget on REE, compared to only 58% for US/UK children. As a result, Shuar AEE was 282 kcal/d (51%) lower than for more sedentary industrialized children when adjusting for age, sex, log-FM, and log-FFM (Fig. 2, Table 1, table S1; $\beta = -0.71$, $SE = 0.18$, $p < 0.001$). Shuar PAL was similarly 0.38 units below that of the US/UK cohort (Fig. 2, Table 1, table S1; $\beta = -0.37$, $SE = 0.06$, $p < 0.001$). Results were consistent in models excluding FM (table S2). Low AEE and PAL among highly active Shuar children indicate that these common measures are not reliable indices of daily physical activity.

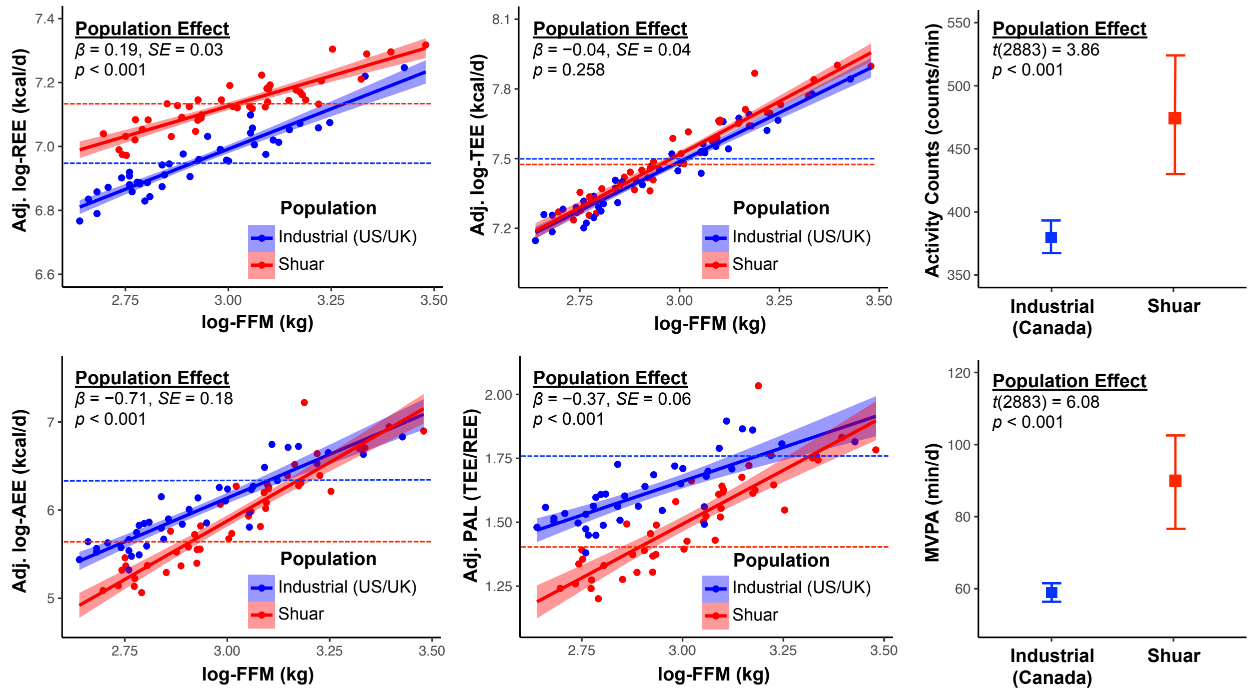

Fig. 2. Energy expenditure and daily physical activity measures for Shuar children (red) and industrialized cohorts (blue). Scatterplot solid lines (shaded 95% confidence intervals) indicate regressions of energetic measures on log-FFM adjusting for age, sex, and log-FM, with dotted lines denoting population estimated marginal means from final energetic models. Plots of accelerometry activity counts and MVPA display unadjusted population means (95% confidence intervals). No population difference was observed for TEE. However, Shuar children had greater REE, lower AEE and PAL, and greater activity counts and MVPA than industrialized references. TEE = total energy expenditure; REE = resting energy expenditure; AEE = activity energy expenditure; PAL = physical activity level; MVPA = moderate-vigorous physical activity; FM = fat mass; FFM = fat-free mass

DISCUSSION

The findings of this study provide evidence for constraint and tradeoffs in energy expenditure during childhood. Shuar forager-horticulturalist children of Amazonia are ~25% more physically active and, in association with elevated immune activity, have ~20% greater REE than children from industrialized populations. Despite these differences, Shuar TEE is indistinguishable from that of US/UK counterparts. These results are consistent with life history theory prediction for adaptive energy allocation and challenge the commonly utilized Additive TEE model of human energy use.

To further investigate the nature of childhood energy constraint and tradeoffs, we modeled the contributions of immune function, growth, body size/composition, and circadian rhythm to TEE and its underlying components for both Shuar and industrialized populations (Fig. 1C; *Materials and Methods*). Elevated Shuar REE does not result from clear differences in FFM composition (i.e., muscle:organ mass ratio; fig. S2) and runs counter to expectation of low REE in tropical climates entailing low thermoregulatory costs (*19*). As demonstrated among other Amazonian forager-horticulturalists (*20*), elevated Shuar REE likely results from persistent immune activation (*7*) in the context of high environmental pathogenicity (*21*). This position is supported in the present sample by a positive relationship between Shuar blood concentration of total Immunoglobulin G

– the most common class of circulating antibody in humans (*22*) – and both log-REE (Fig. 3; $\beta = 0.15$, $SE = 0.05$, $p = 0.003$) and REE elevation above US/UK predicted values (Fig. 3; $\beta = 252.66$, $SE = 64.38$, $p < 0.001$).

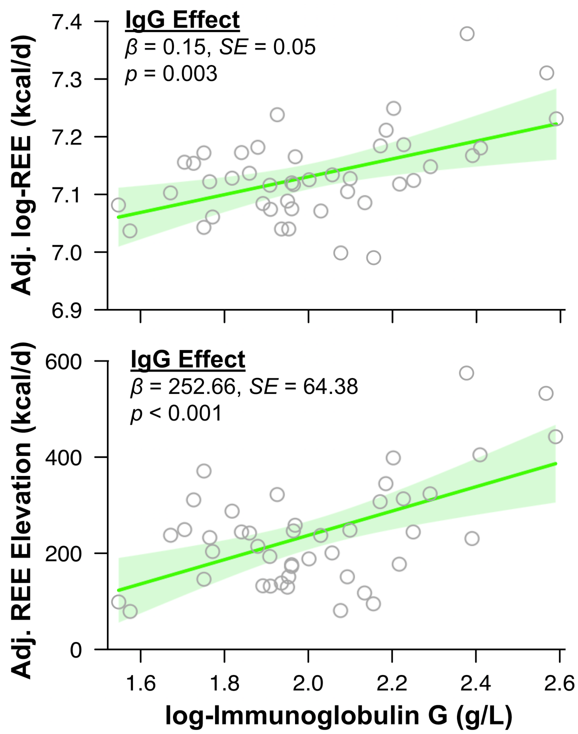

Fig. 3. Shuar total Immunoglobulin G (IgG) concentration versus measured REE (top) and REE elevation above predicted US/UK values (bottom). Solid lines (shaded 95% confidence intervals) indicate regression of log-IgG adjusting for age, sex, log-FM, log-FFM, and time of REE data collection (log-REE analysis) or time of REE data collection (REE elevation analysis). REE elevation was calculated as the difference between measured REE values and REE predicted from the best-fit model of the US/UK sample ($R^2 = 0.588$, $p < 0.001$; adjusting for age, sex, log-FM, and log-FFM). REE = resting energy expenditure; FM = fat mass; FFM = fat-free mass

Shuar children exhibit lower AEE than expected given relatively high accelerometry-measured physical activity. This finding is not readily explained by possible minimal forager-horticulturalist differences in thermic effect of food (owing to dietary differences, *12*; table S3) or by differences in sleep duration (forager-horticulturalist's daily sleep duration is similar to that of industrialized populations, *23*). We modeled three additional factors that could contribute to this finding (*Materials and Methods*). First, 14% greater body mass for the US/UK population (Table 1) will increase the energy cost of movement (i.e., kcal/accelerometer count). Second, Shuar children may be somewhat more energetically efficient in movement owing to greater daily workloads (*24*). Third, circadian fluctuation in REE, which can approach ~10% for adults in industrialized populations (*25*), may be relatively blunted in energetically stressed populations like the Shuar. Consequently, the difference between true 24-hour REE and REE extrapolated from standard early morning measurements may be greater for industrialized populations, thereby inflating their AEE calculation. Modeling the components of TEE with these considerations indicates that differences in immune activity, growth, movement costs, exercise efficiency, and circadian fluctuation in REE can largely explain the observed similarity in Shuar and US/UK TEE (Fig. 1C; 8% error). Unaccounted energy expenditure in the Industrial model may reflect relatively lower metabolic efficiency (e.g., greater mitochondrial proton leak) or greater 24-hour costs of thermoregulation.

Constraint in childhood TEE emphasizes the role of energetic tradeoffs in shaping human developmental plasticity and life history variation. These tradeoffs, while occurring throughout development, are particularly salient during childhood when brain metabolic costs peak (*9*) and often involve physical growth (*7, 8*). Tradeoffs reducing growth result in smaller body size, which decreases energy requirements. Such a response may be adaptive by reducing risk of negative energy balance and starvation in challenging environments. However, it may also have lifetime effects on metabolism (see below) and necessitate extended periods of growth to obtain target adult body size (*26*). Notably, constraint and tradeoffs in childhood energy expenditure imply that models of human life history evolution and cooperative breeding that rely on standard additive estimates of TEE (e.g., *27*) may require restructuring to reliably calculate the energetic cost of supporting dependent offspring in subsistence-based contexts.

Exercise is essential for health in both children and adults (*28*), but evidence for childhood TEE constraint warrants reassessment of the mechanisms linking physical activity and lifetime well-being. Incongruity between lifestyle and TEE at the population level supports the position that secular change in diet (i.e., energy intake), not physical activity (i.e., energy expenditure), is the primary determinant of the chronic energy imbalance underlying increasing global rates of obesity (*29*). For populations in the developing world, TEE constraint implies that elevated physical and immune activity may reduce energy available for childhood growth, even when food is not limited and energy balance is positive. Global disparities in risk of child growth faltering and resulting lifetime metabolic dysregulation (*30*) may therefore be more strongly related to variation in lifestyle and infectious disease burden than variation in energy availability.

The lasting effects of childhood energetic conditions on later life health are well documented (*15*), and metabolic plasticity driven by TEE constraint may contribute to the rapidly emerging dual burden of childhood growth stunting and adult obesity in the developing world (*31*). Future research addressing these topics should build on the limitations of the present work by collecting primary data that span a range of economic development and lifestyle variation within a single transitioning population. Future study should also investigate the nature of energy constraint and tradeoffs across a wider sub-adult age range, including among infants and younger children that are more susceptible to growth faltering, typically in association with infection-induced anorexia and poor nutrient absorption/retention (*32*). Adopting models of childhood energy expenditure that account for constraint and tradeoffs will advance strategies to promote lifetime health.

MATERIALS AND METHODS

Shuar participants and study design

The Shuar are an indigenous population of ≈ 50,000 individuals, many of whom continue to rely on subsistence hunting, fishing, foraging, and horticulture (*33, 34*). The study community of ≈ 300 individuals is located in the isolated cross-Cutucú geographic area.

The community is not accessible by road and has no running water or health clinic. Electricity is limited and highly intermittent. Household-level economic, lifestyle, and dietary information for the study sample are provided in table S3. Data were collected over 14-day study periods during the 2016 annual dry season (October to November). All resident pre-pubertal children (age 5-12 years) were invited to participate, with a final sample of 44 children (generally healthy and non-medicated). No participants were seriously ill at any point during the study, as determined from weekly report by children and their parents, investigator observation, and body temperature assessment (*mean*±*SD* = 98.8 ± 0.5°C, range = 97.8 to 99.9°C). Parental informed consent with child informed assent was obtained from all participants. Study methods and procedures were approved and conducted in accordance with guidelines set by community leaders, the Federación Interprovincial de Centros Shuar, and the Committee on the Use of Human Subjects Institutional Review Boards of University of Oregon and City University of New York.

Anthropometry and daily physical activity

Shuar height (Seca 214 stadiometer, Hanover, MD), weight (Tanita BF-689 scale, Tokyo), triceps skinfold (Beta Technology Lange calipers, Santa Cruz, CA), and arm circumference (Seca 201 tape, Hanover, MD) were measured on Day 0 using conventional methods. Body temperature was measured weekly with an aural thermometer (Welch Allyn Thermoscan Pro 6000, Skaneateles Falls, NY). Physical activity was monitored for a subsample of 30 children over the 14-day study period using Actical triaxial accelerometers (Phillips Respironics, Bend, OR) worn continuously at the right hip. Retrieved data were processed using a macro program to remove non-wear time (*35*), with valid days defined by wear time ≥ 10 hrs. All children had ≥ 5 valid days of data (*mean*±*SD* = 13.8 ± 2.3 days). Activity levels were established using standard child-specific activity count cut points (*36*).

Resting energy expenditure

Shuar REE was measured in triplicate on Days 0, 7, and 14 using a validated Quark RMR respirometry system (COSMED, Rome) with a canopy hood. Five children were measured only twice. Measurements were performed in a quiet room in the supine position, with parents nearby. All measures were made in the morning (*mean*±*SD* = 06:43 ± 43 min), following overnight fast (*mean*±*SD* = 12.4 ± 1.5 hrs reported fast), and prior to strenuous physical activity. Device calibration was performed before each measure using a manufacturer-provided standard gas mixture and volume syringe. Child O_2 consumption and CO_2 production were monitored continuously for ≥ 30 minutes, with the first 10 minutes of data discarded and the remaining steady state period averaged to determine REE using the modified Weir equation (*37*). Overall reliability of repeated weekly REE measures was high (*CV* = 6.4%), with the average of weekly values representing final REE. Model results were similar when analyzing conservative values of Shuar REE that excluded initial or single highest repeated measures (table S4).

Total energy expenditure and body composition

Shuar TEE and body composition were measured using the DLW method (*37*). Oral doses of DLW (6% ^2H_2O, 10% $H_2{}^{18}O$, tailored to body weight) were given to children on Day 0. Urine samples were collected before dosing, ≈ 6 hours post-dose, and on ≈ Days

3, 7, and 11 (*mean*±*SD* assessment = 11.0 ± 1.9 days). Samples were stored at -20°C until measurement of ^{2}H and ^{18}O isotope enrichment via cavity ring-down spectrometry (CRDS; Picarro L2120i, Santa Clara, CA). Isotope depletion rates and dilution spaces were calculated using the slope-intercept method, with rate of CO_2 production subsequently calculated using a two-pool approach and converted to TEE using a food quotient of 0.93, as previously determined for Amazonian forager-horticulturalists (*20*). Due to common dehydration in Amazonian children, fat-free mass (FFM) was calculated using a hydration constant of 0.73. Findings were similar when using a hydration constant of 0.75 (table S5).

Reliability of TEE and body composition measures

The DLW method is the gold standard for the measurement of free-living TEE in humans (*37*). DLW measures of TEE and FFM are directly comparable across labs following standardized data collection and analytical protocols (*37*). To provide additional assurance that our CRDS DLW measures were reliable and directly comparable to US/UK values, duplicate measures were obtained for six Shuar participants using isotope-ratio mass spectrometry at an external lab (table S6; between assay CV_{TEE} = 2.0%, CV_{FFM} = 0.3%).

Total Immunoglobulin G

Shuar circulating concentration of IgG was measured using a validated ELISA protocol in finger-prick dried blood spot (DBS) samples (*7*). Participant DBS were collected following REE measurement on Days 0, 7, and 14 using standard procedures (*38*). All DBS samples were stored at −20°C via a solar-powered freezer until shipped to the US for storage at −30°C. Samples were measured for IgG in duplicate with two-level controls. Intra- and inter-assay measurement coefficients of variation (CVs) for the assay are 2.0-2.7% and 8.0-10.8%, respectively (*7*). Final IgG concentration was obtained by averaging participant repeated weekly measures.

Comparative industrialized cohorts

Energetics data for the US/UK cohort were obtained from studies of healthy pre-pubertal children and were selected for their use of similar coupled DLW/respirometry protocols and complete individual-level data. Data were included for 20 children (age 5-6 years) from VT, USA (*16*) and 20 children (age 7-9 years) from Northern Ireland, UK (*17*). One child from the published US sample was excluded due to negative calculated AEE. Similar to the Shuar sample, TEE was measured over a ≈ 2-week period and REE was measured in the morning prior to any strenuous activity using a canopy hood. Measures of REE equivalent to those obtained for the Shuar were reported as 'basal metabolic rate' in the UK study. For US children only, reported non-fasted REE values were converted to fasted REE by multiplying by 0.89, a published correction factor determined and validated specifically for the US study protocol (*39*). This had minimal effect on the analysis, as Shuar REE remained significantly greater (+169 kcal/d) than that of the US/UK cohort even when modeling uncorrected (i.e., inflated) US REE values (β = 0.14, *SE* = 0.03, $p < 0.001$). US and UK energetic measures (i.e., TEE, REE, AEE) did not significantly differ at the group level (all $p < 0.1$), and TEE and REE measures were similar to values calculated by common prediction equations (table S7). This supports the treatment of the US and UK cohorts as a single, broadly representative industrialized cohort in GLM models (see below).

Accelerometry data are not available for the US/UK cohort. As such, physical activity data for a nationally representative Canadian cohort (Canadian Health Measures Survey 2009-2015; age 5-12 years, $N = 1433$ males and $N = 1422$ females) were used (*18; Data S2*). These data were collected with Actical devices and were processed using similar methods to those for the Shuar. Canadian cohort MVPA (58 min/d) approximates that reported for the UK cohort (64 min/d) measured by the flex-heart rate method (*17*). This finding suggests that physical activity patterns are broadly similar across the two groups. Canadian physical activity measures are also comparable to those reported for US NHANES references (*40*), suggesting general agreement in daily physical activity across the Canadian, UK, and US cohorts.

Data analysis

Participant AEE was calculated as TEE − (REE + 0.1TEE), where 0.1TEE reflects thermic effect of food (TEF; *12*). Population differences in daily physical activity measures were tested using two-sample *t*-tests. Population differences in anthropometric and energetic measures were tested using generalized linear models, following conventional methods. Age, sex, log-FM, and log-FFM were included as predictors in all final energetic models. Body mass and BMI were examined in preliminary analyses, with model fit assessed using residual sum of squares. Details for specific models are provided in figure and table captions. Post hoc diagnostic analyses revealed acceptable degrees of linearity, heteroscedasticity, and multicollinearity in final models. All analyses were performed in R (cran.us.r-project.org/), with results reported as statistically significant at $p < 0.05$.

Modeling TEE component energy budgets

To investigate the contribution of different metabolic tasks to child TEE in Shuar and industrialized populations, we modeled energy utilized in REE, diurnal REE fluctuation, physical activity, immune function, growth, and digestion.

REE: Human REE is strongly related to FFM, specifically the size and tissue-specific metabolic rates of the internal organs (*41*). As the FFM of Shuar and US/UK children were indistinguishable (Table 1), we used the observed REE value for the US/UK sample (1,042 kcal/d, Table 1) for both the Shuar and Industrial TEE models.

Diurnal REE fluctuation: REE fluctuates throughout the day in a circadian rhythm, with its nadir near 06:00 and its peak near 16:00 (*25*). The mean diurnal difference between minimum and maximal adult REE is ~10% (*25*). We modeled an additional 10% increment of REE for the Industrial TEE model (diurnal REE fluctuation increment = 0.1REE, or 104 kcal/d). We hypothesize that diurnal fluctuation in REE is related to the diurnal production of regulatory metabolic hormones (e.g., cortisol, testosterone). In subsistence populations like the Shuar, and similarly in physically active populations, waking and diurnal salivary cortisol levels are reduced as much as ~80% compared to industrialized populations (*42*). Thus, we modeled only a 2% increment in REE for the Shuar TEE model (diurnal REE fluctuation increment = 0.02REE, or 21 kcal/d).

Immune function: Given the positive relationship between blood markers of immune activity and REE in both the present Shuar sample (Fig. 3) and in other Amazonian forager-horticulturalist populations (*20*), we assumed that the elevation of Shuar children's REE relative to US/UK values was entirely due to greater infectious disease burden and resultant immune activity. This approach yields an immune function cost for Shuar children of 192 kcal/d. This estimate is similar to that reported for adult Tsimane, an Amazonian population that is ecologically and immunologically similar to the Shuar (*7, 20*).

Growth: Growth costs for the Industrial TEE model were estimated by multiplying the mean rate of growth for US children age 3-10 years old (7.2 g/d; *26, 43*) by the childhood-specific cost of synthesizing all new tissue (1.8 kcal/g; *12*). This cost reflects the energy needed to synthesize new tissue and does not include the energy content of the new tissue itself, which is not captured in DLW measures of TEE. The cost of synthesizing new tissue for growth is thus 13 kcal/d for the Industrial TEE model. Based on ~20% slower growth velocities among Shuar children (*26*), we estimated the growth cost for the Shuar TEE model as 10 kcal/d.

Physical activity: We estimated the energy cost of physical activity using the ratio of AEE to accelerometer-measured body movement among the Shuar sample. This approach assumes that AEE among the Shuar is entirely (or almost entirely) reflective of musculoskeletal activity, whereas AEE for the US/UK cohort is inflated by greater diurnal fluctuation in REE. For Shuar children, the ratio of AEE/mean accelerometer counts per minute (CPM, Table 1) yields 0.58 kcal/CPM. Mean body mass in the US/UK sample is 14% greater than the Shuar sample (Table 1), indicating that that the cost of movement for US/UK children should be 14% greater, or 0.66 kcal/CPM. Finally, we assumed that the efficiency of movement might be up to 5% greater for the Shuar due to their greater habitual physical activity (*24*), which yields a final US/UK cost of movement of 0.70 kcal/CPM. Multiplying this ratio by observed mean CPM for the Industrial sample (379 CPM) yields a cost of physical activity of 264 kcal/d for the Industrial TEE model.

Digestion: The energy cost of digestion (TEF) is closely related to TEE and was estimated as 0.1TEE (*12*) in both the Shuar and Industrial TEE models.

SUPPLEMENTARY MATERIALS

fig. S1. Analysis of TEE measures for Shuar children and expanded industrialized cohorts
fig. S2. Shuar arm muscle area measures as percentiles of US references
table S1. Parameter estimates for final energetics models
table S2. Parameter estimates for energetics models that do not include FM as a predictor
table S3. Household-level lifestyle, economic, and dietary information for the Shuar sample
table S4. Parameter estimates for energetics models evaluating conservative values of Shuar REE
table S5. Parameter estimates for energetics models using a hydration constant of 0.75
table S6. Validation of Shuar TEE and FFM measures against isotope ratio mass spectrometry
table S7. Measured REE and TEE for US/UK children compared to predicted values
data S1. Primary study data with variable list
data S2. Daily physical activity summary data for the Canadian cohort
data S3. Expanded industrialized sample data

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Supplementary Materials for

Constraint and Tradeoffs Regulate Energy Expenditure During Childhood

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This PDF file includes:

Figs. S1 and S2
Tables S1 to S7
Captions for Data S1 to S3

Other Supplementary Materials for this manuscript include the following:

Data S1 to S3 (Excel format)

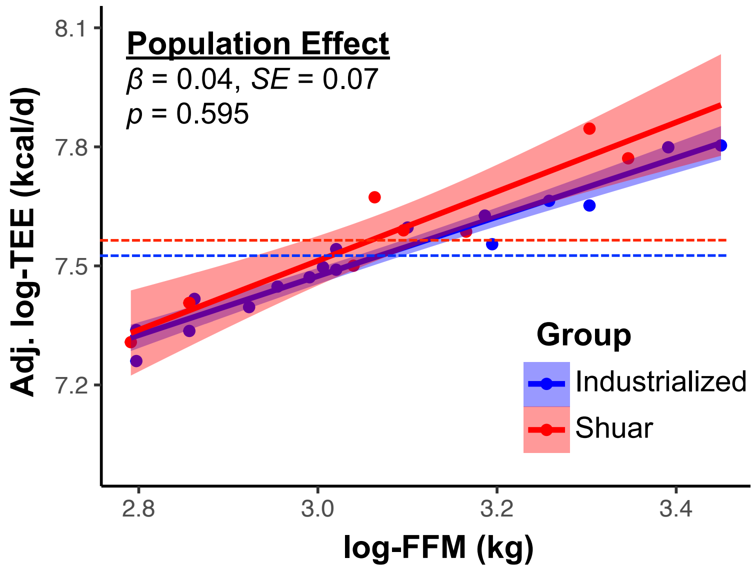

Fig. S1. TEE measures for Shuar children (red) and a larger and more diverse sample of industrialized cohorts (blue). Solid lines (shaded 95% confidence intervals) indicate regression of log-TEE on log-FFM adjusting for age, sex, and log-FM. Dotted lines denote group estimated marginal means from the final model that included log-FFM. No group difference was found in log-TEE ($\beta = 0.04$, SE = 0.07, $p = 0.595$). Data points represent sample-level mean values (binned by 2-year age and sex groups for Shuar and reported 1 to 5-year age and sex groups for industrialized cohorts). Industrialized cohorts (N = 17; N = 336 children; age 5-10 years; healthy and normal weight) were drawn from the US (16, 44, 45), UK (17), and Australia (46, 47). Data and sample details are provided in Data S3. TEE = total energy expenditure; FM = fat mass; FFM = fat-free mass

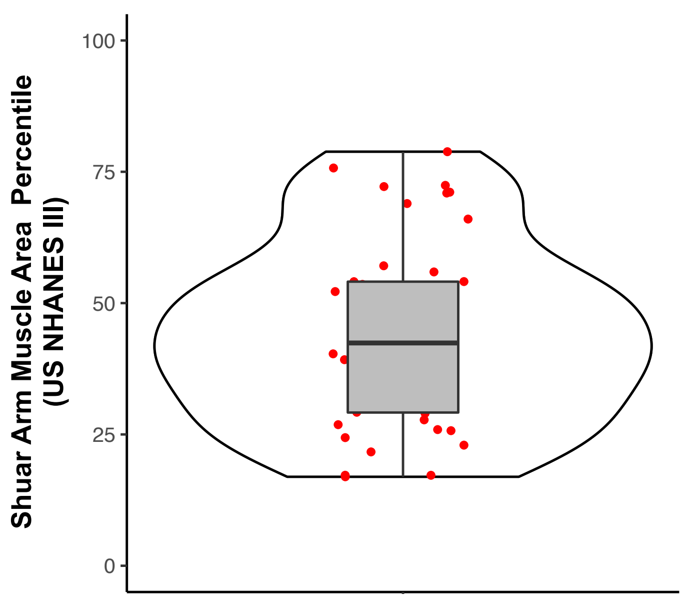

Fig. S2. Shuar arm muscle area (AMA) measures as percentiles of US age- and sex-matched references (NHANES III). Shuar mean AMA is equivalent to the US 44th percentile, indicating skeletal muscle mass approximating that of industrialized children. No difference in total FFM was observed between Shuar and US/UK children (Table 1), suggesting similar fat-free mass composition (i.e., skeletal muscle:organ mass ratio). Shuar AMA was calculated from arm skinfolds and circumference measures (48). Boxplot denotes 25th, 50th, and 75th quantiles.

Table S1. Parameter estimates [*β* (*SE*)] for final energetics GLM models.

	Model			
	log-REE (kcal/d)	log-TEE (kcal/d)	log-AEE (kcal/d)	PAL
Intercept	5.45 (0.19)***	4.70 (0.24)***	−0.66 (1.23)	−0.31 (0.43)
Age (yrs)	−0.02 (0.01)*	0.01 (0.01)	0.09 (0.05)	0.04 (0.02)*
Sex (male)	0.07 (0.02)**	0.04 (0.03)	−0.06 (0.13)	−0.03 (0.05)
log-FM (kg)	0.05 (0.03)	−0.09 (0.04)*	−0.52 (0.19)**	−0.24 (0.07)***
log-FFM (kg)	0.53 (0.09)***	0.96 (0.11)***	2.35 (0.56)***	0.70 (0.20)***
Population (Shuar)	0.19 (0.03)***	−0.04 (0.04)	-0.71 (0.18)***	-0.37 (0.06)***
Model adjusted. r^2	0.713	0.768	0.467	0.461

* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$

Table S2. Parameter estimates [*β* (*SE*)] for energetics GLM models that do not include FM as a predictor. Results were consistent with final models.

	Model			
	log-REE (kcal/d)	log-TEE (kcal/d)	log-AEE (kcal/d)	PAL
Intercept	5.32 (0.18)***	4.94 (0.23)***	0.71 (1.18)	0.31 (0.43)
Age (yrs)	−0.02 (0.01)**	0.01 (0.01)	0.11 (0.05)*	0.05 (0.02)*
Sex (male)	0.05 (0.02)**	0.07 (0.02)**	0.11 (0.12)	0.04 (0.04)
log-FFM (kg)	0.60 (0.08)***	0.81 (0.10)***	1.52 (0.50)**	0.32 (0.18)
Population (Shuar)	0.15 (0.02)***	0.03 (0.02)	−0.31 (0.12)*	−0.19 (0.04)***
Model adjusted. r^2	0.707	0.752	0.421	0.379

* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$

Table S3. Household-level lifestyle, economic, and dietary information for the Shuar study sample (*N* = 18 households).

Lifestyle and Economic Variables (% of total or mean [SD])	
Household size (# individuals)	7.4 (2.4)
Household member hunts (frequency/week)	2.1 (2.0)
Household member fishes (frequency/week)	5.4 (2.2)
Household member forages (frequency/week)	2.8 (1.6)
Income (total USD/month)	32 (31)
Dirt-floor home (vs. wood plank, %)	22%
Have running water (%)	0%
Boil water before drinking (%)	0%
Have latrine (%)	33%
Cook with wood (%)	94%
Sleep directly on floor (%)	39%
Sleep using mosquito net (%)	39%
Have light bulb (%)	94%
Dietary Variables (mean [SD])	
Consume garden item (frequency/week)	33.5 (4.5)
Consume hunted item (frequency/week)	1.3 (1.8)
Consume fished item (frequency/week)	3.7 (2.0)
Consume market carbohydrate item (frequency/week)	2.4 (3.6)
Consume market fat/sugar item (frequency/week)	3.0 (3.4)
Consume market protein item (frequency/week)	1.1 (2.0)

Market carbohydrate item = rice, pasta, bread; Market fat/sugar item = cooking oil, soda, potato chips, butter, cookies, sweets; Market protein item = beef, pork, milk

Table S4. Parameter estimates [β (SE)] for GLM models evaluating conservative values of Shuar REE that excluded initial (REEi) or single highest (REEh) repeated weekly measures. Results were consistent with final models.

	Model	
	log-REEi (kcal/d)	log-REEh (kcal/d)
Intercept	5.42 (0.20)***	5.41 (0.20)***
Age (yrs)	−0.02 (0.01)**	−0.02 (0.01)*
Sex (male)	0.07 (0.02)**	0.07 (0.02)**
log-FM (kg)	0.06 (0.03)	0.05 (0.03)
log-FFM (kg)	0.53 (0.09)***	0.54 (0.09)***
Population (Shuar)	0.17 (0.03)***	0.15 (0.03)***
Model adjusted. r^2	0.681	0.669

* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$

Table S5. Parameter estimates [β (SE)] for GLM models using an alternative hydration constant of 0.75 for Shuar and US cohort FM and FFM calculation. Results were consistent with final models.

	Model			
	log-REE (kcal/d)	log-TEE (kcal/d)	log-AEE (kcal/d)	PAL
Intercept	5.46 (0.20)***	4.77 (0.26)***	−0.54 (1.30)	−0.21 (0.46)
Age (yrs)	−0.02 (0.01)*	0.01 (0.01)	0.11 (0.05)*	0.05 (0.02)**
Sex (male)	0.07 (0.02)**	0.05 (0.03)	−0.04 (0.13)	−0.02 (0.05)
log-FM (kg)	0.06 (0.03)	−0.08 (0.05)	−0.53 (0.22)*	−0.23 (0.08)**
log-FFM (kg)	0.52 (0.09)***	0.92 (0.12)***	2.30 (0.60)***	0.64 (0.21)**
Population (Shuar)	0.19 (0.03)***	−0.01 (0.04)	−0.63 (0.18)***	−0.33 (0.07)***
Model adjusted. r^2	0.714	0.748	0.448	0.426

* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$

Table S6. Measured TEE and FFM using cavity ring-down spectrometry and duplicate measures (TEEirms; FFMirms) obtained for six participants using isotope ratio mass spectrometry. Results demonstrate between-method reliability.

	TEE (kcal/d)	TEEirms (kcal/d)	TEEdif (kcal/d)	TEEdif (%)	FFM (kg)	FFMirms (kg)	FFMdif (kg)	FFMdif (%)
Child 1	1819	1978	-159	8.0	22.3	22.5	-0.1	0.7
Child 2	1973	1935	38	2.0	23.5	23.9	-0.4	1.7
Child 3	2052	2034	18	0.9	27.9	28.2	-0.3	1.1
Child 4	2209	2478	-269	10.9	24.1	24.4	-0.3	1.2
Child 5	1514	1403	111	7.9	15.9	15.9	0.0	0.0
Child 6	1678	1729	-51	2.9	18.1	18.3	-0.2	1.1
Mean (SD)	*1874(255)*	*1926 (355)*	*-52 (140)*	*5.4 (4.0)*	*22.0 (4.3)*	*22.2 (4.4)*	*-0.2 (0.1)*	*1.0 (0.6)*

Table S7. Measured REE and TEE for US/UK children and predicted values calculated by common prediction equations (that were developed using predominantly industrialized samples). Small differences between measured and predicted values support the treatment of the US and UK cohorts as generally representative of industrialized children.

	Measured† (kcal/d)	Predicted‡ (kcal/d)	Difference (kcal/d)	Difference (%)
REE	1057	1027	30	2.8
TEE	1719	1651	68	3.9

†Unadjusted values; ‡Predicted values for REE were calculated using the childhood-specific equations of Schofield (49; based on sex, age, weight, and height). Predicted values for TEE were calculated using the childhood-specific WHO equations (50; based on sex and quadratic weight).

Data S1. Primary study data with variable list

Data S2. Daily physical activity summary data for the Canadian cohort

Data S3. Expanded industrialized sample data