Leukocyte telomere length (LTL) in the Costa Rican Longevity and Healthy Aging Study (CRELES):

Correlates, measurement issues, longitudinal change, and prospective mortality

April 25, 2018

Luis Rosero-Bixby, University of Costa Rica
David H. Rehkopf, Stanford University
William H. Dow, University of California at Berkeley
Elissa Epel, University of California, San Francisco
Jue Lin, University of California, San Francisco
Elizabeth H. Blackburn, University of California, San Francisco

Funding support from NIA P30-AG012839 and K01-AG047280

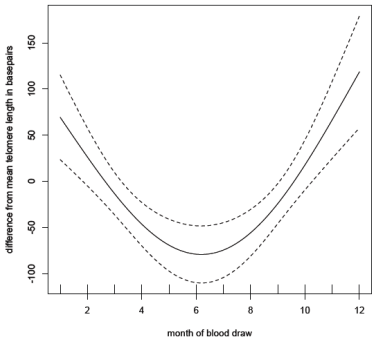
CRELES advantages for LTL research

- LTL associated with SES, stress, smoking, CVD risk, mortality, etc. But some limitations:
  - Results not uniformly found across studies. Furthermore, most large studies are from US/Europe, with rare exceptions (such as Taiwan).
    - Generalizable to other settings such as Costa Rica, with potentially different confounding structures (e.g., flat SES-health gradients)?
  - Most studies have only one measure of LTL, so analyze only cross-sectional associations.
    - CRELES has two measures, ~2 years apart

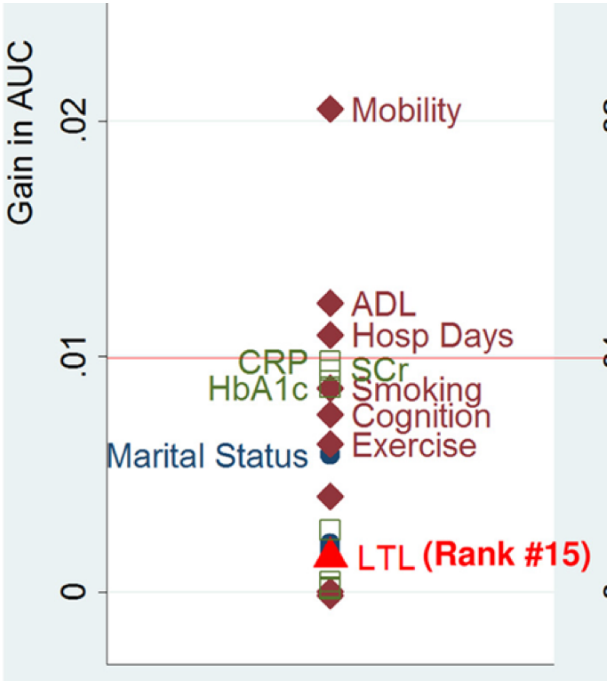
Prior telomere findings in CRELES

1. Longer telomere length in Nicoyan Blue Zone (Rehkopf et al., Exp Gerontol, 2013).

2. First documentation of strong seasonality associated with telomere length, possibly tied to rainy season (Rehkopf et al., A J Human Bio, 2014



3. Age and many standard risk factors are much stronger predictors of mortality than is LTL (Glei et al., PLoS One, 2016).



Further CRELES LTL study questions, exploiting repeated LTL measure (in newly expanded sample)

- LTL association with demographics, SES, health outcomes, biomarkers, and early childhood conditions
- Effect of blood & DNA storage time on LTL measures
- LTL association with prospective survival

CRELES Data

- Probabilistic national sample of 2,827 adults age 60+, selected from the 2000 Census. Oversampled oldest-old.
  - Supplemental sample from Nicoyan “Blue Zone.”
- Household visits in 2005, 2007, 2009.
  - Mortality follow-up ongoing in National Death Index.
- Extensive biomarkers (95% participation) in 2005 and 2007, including fasting venous blood.
- LTL in nested subsample with blood:
  - 100% of Nicoyan sample and 38% from rest of country, age stratified.
  - 1,299 participants, 968 with two LTL measures.
    - Roughly 2 years apart, but within-person seasonal variation.

DNA extraction, blood storage, and LTL assay

- University of Costa Rica lab extracted DNA from either fresh blood or 2ml of frozen whole blood cells, using phenol-chloroform method.

- DNA samples, with mean concentration of 106 ng/ul, stored in Costa Rica at 4 degrees Celsius for 0-9 years.
  - Samples then shipped to San Francisco on dry ice, then stored at -80 celsius.

- Quantitative PCR assay used to determine measure of telomere length: relative ratio of telomere repeat copy number to single-copy gene copy number (t/s ratio).
  - Two assays per DNA sample were averaged (inter-assay coefficient of variation=3.7%)

- Assays conducted in two batches: 2010 and 2014
  - 29 samples from 2010 re-assayed in 2014. Correlation=0.94, but mean 2014 T/S ratio 0.07 longer (~8%) in this validation sample.

Covariates

1. *Measurement procedures*: years DNA stored at 4 degrees C, DNA was extracted from fresh blood, season when blood was collected, and 2010 v 2014 batch.

2. *Demographic and SES*: age, gender, time to death, Nicoyan, widowed, living alone, education, income.

3. *Health outcomes*: self reported GHS, cancer, diabetes, anti-hypertensives, ADLs, mini-mental, depression.

4. *Biomarkers*: systolic and diastolic BP, BMI, grip strength, total/HDL cholesterol, triglycerides, CRP, HbA1c, serum creatinine.

5. *Childhood conditions*: knee height, GHS, malaria, asthma, economic hardships.

Descriptive Statistics

Variables	Units	Mean	(S. Err.)	Valid N	Missing
Telomere length T/S ratio	T/S ratio	0.923	(.007)	2,229	0
Measurement factors					
Assay lot 1	Binary 0-1	0.381	(.022)	2,229	0
March-May	Binary 0-1	0.209	(.012)	2,229	0
October-December	Binary 0-1	0.302	(.017)	2,229	0
DNA storage time	N. years	4.987	(.091)	2,229	0
Fresh blood cells	Binary 0-1	0.445	(.018)	2,229	0
Demographics					
Exact age	Years	71.628	(.309)	2,229	0
Deceased in < 3 yrs	Binary 0-1	0.127	(.017)	2,229	0
Deceased in 3-5 yrs	Binary 0-1	0.117	(.013)	2,229	0
Gender = male	Binary 0-1	0.462	(.022)	2,229	0

Regression associations with LTL:
* Confirm longer for younger, females, Nicoyan
* SES not significantly related (with controls)

Explanatory factors	Base model (N variable)			Full model RE (N=2229)			Full model FE (N=930)	
Demographic & SES								
Exact age in years	-.0051	(.0007)	**	-.0042	(.0006)	**	-.0010	(.0077)
Deceased in < 3 yrs.	.0219	(.0247)		.0128	(.0129)		-.0225	(.0294)
Deceased in 3-5 yrs.	-.0157	(.0163)		-.0066	(.0110)		-.0112	(.0202)
Gender = male	-.0273	(.0133)	*	-.0479	(.0129)	**		
Nicoya region	.0258	(.0179)		.0300	(.0114)	**		
Widow	-.0045	(.0135)		.0020	(.0098)		-.0151	(.0233)
Living alone	-.0003	(.0150)		-.0057	(.0120)		-.0084	(.0207)
Education years	-.0012	(.0018)		.0002	(.0014)			
Income	-.0034	(.0012)	**	-.0015	(.0009)		-.0005	(.0012)

** p<.01, * p<.10
Base: OLS model of each variable separately, controlling only age & sex
Full: all controls
RE: random effects
FE: fixed effects

Diabetes diagnosis is only significant health marker (a few marginally significant markers)

Health									
Reported poor health	.0134	(.0064)	*	.0008	(.0040)		-.0019	(.0055)	
Smoker	-.0338	(.0244)		-.0119	(.0165)		.0265	(.0353)	
Cancer diagnosed	.0560	(.0284)	*	-.0009	(.0177)		.0270	(.0480)	
Diabetes diagnosed	-.0084	(.0163)		-.0297	(.0119)	**	-.0226	(.0301)	
Taking BP medicine	-.0070	(.0128)		-.0006	(.0082)		.0013	(.0156)	
ADLs disability	.0006	(.0003)	*	.0000	(.0002)		.0002	(.0004)	
Cognition impairment	.0010	(.0006)	*	-.0001	(.0004)		-.0011	(.0006)	*
Depression symptoms	.0005	(.0003)	*	.0002	(.0002)		.0000	(.0003)	
Biomarkers									
Systolic BP	.0002	(.0002)		.0004	(.0002)	*	.0001	(.0003)	
Diastolic BP	.0004	(.0005)		-.0001	(.0004)		.0002	(.0006)	
BMI	-.0005	(.0012)		-.0003	(.0008)		.0017	(.0022)	
Hand grip strength	-.0007	(.0012)		.0004	(.0008)		.0021	(.0012)	*
Total/HDL choles. ratio	-.0066	(.0041)		-.0019	(.0027)		-.0012	(.0043)	
Triglycerides	-.0001	(.0001)		.0000	(.0001)		-.0001	(.0001)	
CRP	.0022	(.0007)	**	.0007	(.0005)		.0012	(.0007)	*
HbA1c	.0002	(.0048)		.0055	(.0037)		.0070	(.0057)	
Serum creatinine	-.0129	(.0147)		-.0200	(.0115)	*	-.0273	(.0237)	
DHEAS	.0001	(.0002)		.0002	(.0001)	*	.0002	(.0002)	

Early childhood markers not related to LTL (knee height marginally significant)

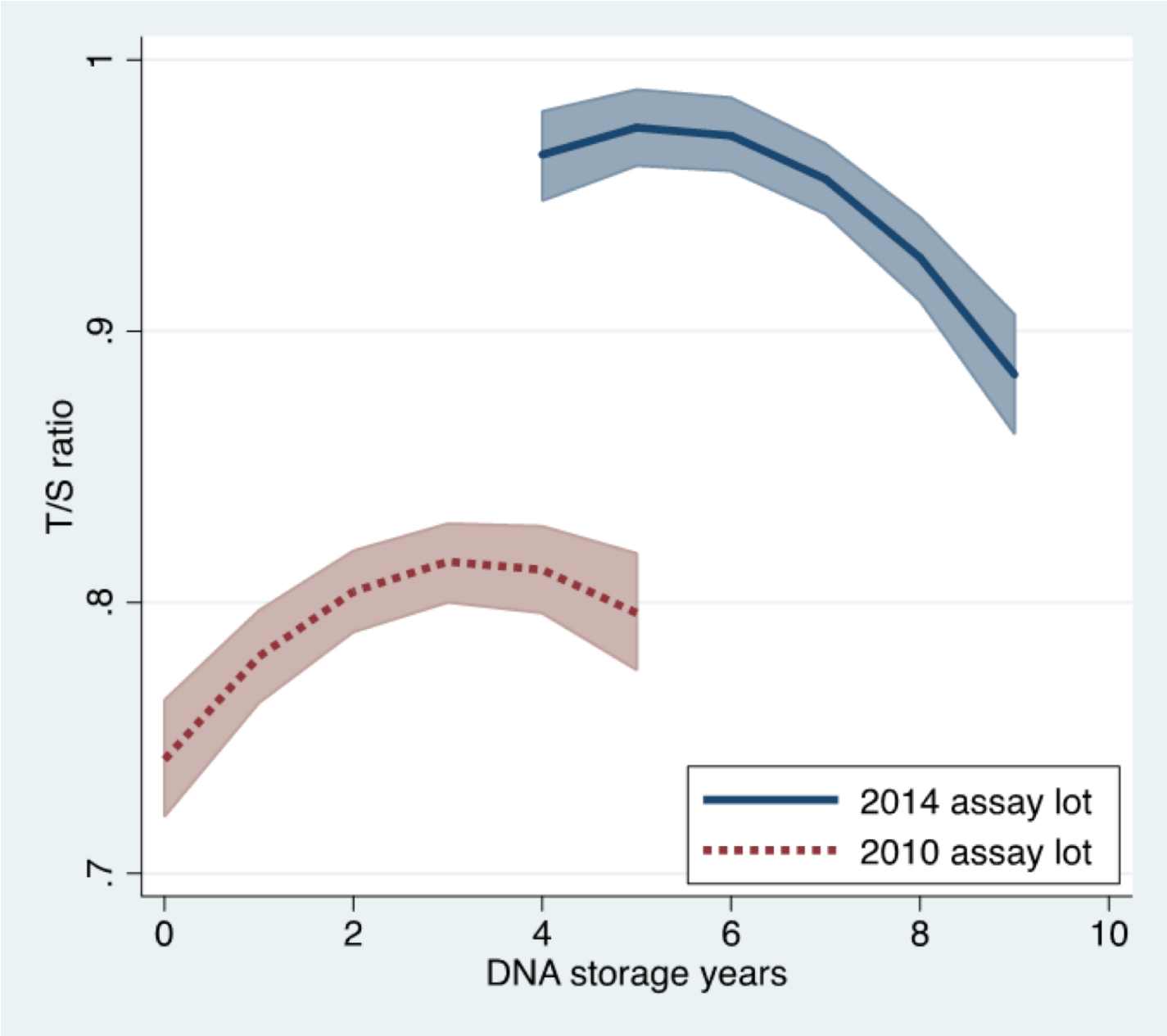
Early childhood					
Knee height	.0024	(.0025)	.0028	(.0018)	*
Childhood poor health	-.0021	(.0071)	-.0010	(.0051)	
Childhood malaria	-.0036	(.0215)	-.0053	(.0163)	
Childhood asthma	-.0101	(.0269)	.0055	(.0168)	
Childhood hardship	-.0153	(.0229)	-.0010	(.0159)	

Storage & timing factors are strongly related to LTL measurement

* confirm seasonality finding (~=6 years of aging)
* later assay lot (~=10 years of aging)
* fresh blood cells (~=7 years of aging)
* years stored has non-linear association:
  … 4 years storage ~= 10 years of age younger
  … but longer storage ~= 10 years of age older

Explanatory factors	Base model & (N variable)			Full model RE (N=2229)			Full model FE (N=930)		
October-December	.0227	(.0135)	*	.0327	(.0077)	**	.0418	(.0100)	**
March-May	-.0329	(.0147)	*	-.0156	(.0091)	*	-.0107	(.0124)	
Assay lot 2010	.0066	(.0598)		-.0513	(.0367)		-.1361	(.0519)	**
Fresh blood cells	.0021	(.0156)		.0393	(.0086)	**	.0539	(.0170)	**
Years DNA stored	.0859	(.0201)	**	.0694	(.0124)	**	.0500	(.0148)	**
Years DNA squared	-.0078	(.0016)	**	-.0067	(.0010)	**	-.0050	(.0014)	**
Lot * storage interact.	-.0386	(.0125)	**	-.0263	(.0084)	**	-.0109	(.0114)	

Nonlinear marginal effects of storage time



Why is LTL (nonlinearly) related to storage time??

- Lengthening:
  - Residual proteins break down, unfolding the DNA and thus exposing a longer telomere template?
  - But extended denaturation step before PCR should unfold all DNA.

- Shortening:
  - Non-sterile storage allows bacterial and fungal nucleases that can degrade LTL?
  - But ran gels on N=110, and only 5 showed degradation (and were discarded).

- Other concerns, but unclear why time-dependent:
  - Oxidation of DNA during storage may impact PCR reactions.
  - Residual chemicals from DNA extraction could influence PCR.

Discussion: Costa Rican LTL …

- Strongly related to age, sex, seasonality, Nicoyan.

- Weakly related to SES, health and early childhood conditions, despite large N.
  - Only diabetes diagnosis relation is strong.
  - Possibly CRP and income, if models are over-controlling?
  - Ongoing work: weak prediction of mortality

- Strongly related to storage time.
  - Is measurement noise from storage masking effects, counteracting benefits of large sample?
    - But: adjusting for measurement has little effect on estimated associations
  - Note correlation between 2 measures two years apart is 0.57 … so there is substantial volatility, but unclear if due to measurement or natural variability.

Normalization of T/S ratio based on random effects model coefficients

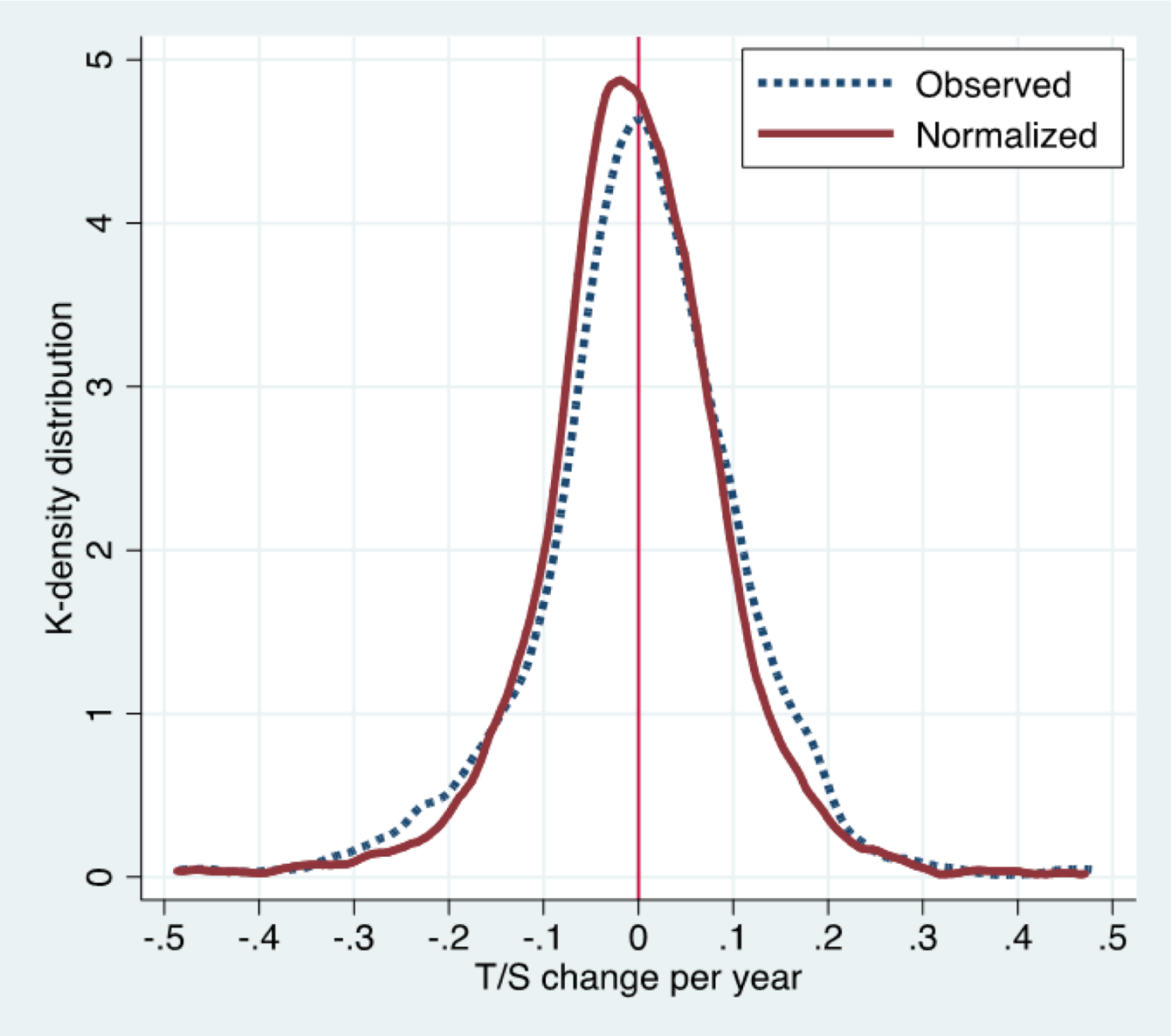
2014 LTL assay:

$$\text{Adj\_T/S} = \text{Obs\_T/S} - (.069\ \text{DNAyrs} - .0067\ \text{DNAyrs}^2) + .04\ \text{oldblood}$$

2010 LTL assay:

$$\text{Adj\_T/S} = \text{Obs\_T/S} - (.043\ \text{DNAyrs} - .0067\ \text{DNAyrs}^2) + .04\ \text{oldblood} + .051$$

Density of annual change in LTL



Gender – male	Binary 0-1	0.482	(.022)	2,229	0
Nicoya region	Binary 0-1	0.080	(.007)	2,229	0
Widow	Binary 0-1	0.212	(.015)	2,226	3
Living alone	Binary 0-1	0.105	(.012)	2,210	19
Education years	Years	5.440	(.219)	2,229	0
Income	Colon 100000	2.21	(.188)	2,201	28
Health					
Self reported poor health	Scale 1-5	3.270	(.039)	2,225	4
Smoker	Binary 0-1	0.091	(.013)	2,229	0
Cancer diagnosed	Binary 0-1	0.052	(.010)	2,209	20
Diabetes diagnosed	Binary 0-1	0.234	(.019)	2,209	20
Taking BP medicine	Binary 0-1	0.455	(.021)	2,229	0
ADLs disability	Scale 0-100	17.427	(1.018)	2,227	2
Cognition impairment	Scale 0-100	11.762	(.493)	2,226	3
Depression symptoms	Scale 0-100	17.189	(.826)	1,477	752
Biomarkers					
Systolic BP	mmHg	143.981	(.949)	2,208	21
Diastolic BP	mmHg	83.094	(.487)	2,208	21
BMI	Kg/m2	26.509	(.226)	2,204	25
Hand grip strength	kg	32.252	(.304)	1,980	249
Total/HDL cholesterol	ratio	5.045	(.057)	2,211	18
Triglycerides	mg/dl	162.858	(3.590)	2,209	20
CRP	mg/l	5.657	(.386)	2,185	44
HbA1c	percent	5.975	(.056)	2,193	36
Serum creatinine	mg/dl	0.979	(.016)	2,213	16
DHEAS	ug/dl	52.538	(1.831)	2,197	32
Early childhood					
Knee height	cm	49.392	(.143)	2,226	3
Childhood poor health	Scale 1-4	2.221	(.045)	1,550	679
Childhood malaria	Binary 0-1	0.094	(.013)	1,550	679
Childhood asthma	Binary 0-1	0.110	(.015)	1,544	685
Childhood hardship	Scale 0-1	0.494	(.016)	1,556	673