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Original Article

The low male voice is a costly signal of phenotypic quality among Bolivian adolescents

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ABSTRACT

The human voice is one of the most conspicuous and dimorphic human secondary sexual characteristics; males' low fundamental and formant frequencies barely overlap with females'. Researchers often assert that low male voices are costly signals of phenotypic quality; however, no evidence currently exists linking low voices with indicators of quality (i.e., health or physical condition) during the ages where the larynx develops to adult proportions. In the present study, we examine the relationships between condition, testosterone, and vocal parameters in 91 Bolivian peri-pubertal adolescent males. Condition is operationalized as immune function (based on secretory IgA) and energetic reserves (BMI-for-age residuals from Tsimane-specific growth curves, and body fat percentage), and "masculine" vocal parameters is operationalized as having low fundamental frequency, narrow formant position, and low fundamental-frequency variation. We target peri-pubertal individuals to capture variation in vocal parameters during the canalization period for vocal fold and vocal tract growth. Results indicate that males in better energetic condition have higher testosterone levels and lower voices, controlling for age. Further, testosterone mediates the relationship between condition and a lower voice (i.e., lower fundamental and formant frequencies). We suggest that testosterone plays a key mediating role in the causal pathway linking phenotypic condition to a "masculine" voice. Our results provide support for a costly-signal model of low men's voices.

1. Introduction

Secondary sexual characteristics facilitate male competitive and courtship interactions in a wide variety of vertebrates (Andersson, 1994). Among humans, one of the most conspicuous and dimorphic secondary sexual characteristics is the voice. Males have substantially lower fundamental and formant frequencies than women (Baken, 1987; Puts, 2005; Titze, 1994) due to testosterone-driven vocal fold enlargement and vocal tract lengthening during male puberty (Fitch & Giedd, 1999; Kahane, 1978; Harries, Hawkins, Hacking, & Hughes, 1998; Vuorenkoski, Lenko, Tjernlund, & Vuorenkoski, 1978). Lower male voices are perceived as belonging to men who are older (Collins, 2000; Feinberg, Jones, Little, Burt, & Perrett, 2005), larger (Collins, 2000; Feinberg et al., 2005), more physically and socially dominant (Puts, Gaulin, & Verdolini, 2006; Puts, Hodges, Cárdenas, & Gaulin, 2007; Sell et al., 2010; Wolff & Puts, 2010), and more attractive to women (Collins, 2000; Feinberg et al., 2005; Puts, 2005). These perceptions carry important reproductive, social, economic, and political consequences (Apicella, Feinberg, & Marlowe, 2007; Hodges-Simeon, Gaulin, & Puts, 2010; Klofstad, Anderson, & Peters, 2012; Tigue, Borak, O'Connor, Schandl, & Feinberg, 2012).

According to Hamilton and Zuk (1982), observers attend to conspicuous sexually dimorphic traits like the voice because they provide important information about the condition or quality of the speaker. In this model, quality- or condition-dependent characteristics provide conspecifics with honest signals of the bearer's genetic resistance to parasites due to the costs involved in building and maintaining the display trait (Zahavi, 1975). Folstad and Karter (1992) later proposed a proximate mechanism for this model; according to their 'immunocompetence handicap hypothesis' only males who are highly immunocompetent can tolerate the high testosterone (T) levels required to fully express their conspicuous characters. Experimental manipulation of T in animal models shows that high levels of T down-regulate humoral and cell mediated immune function (Duffy, Bentley, Drazen, & Ball, 2000) further supporting the idea that T imposes an immunosuppressant burden that only can be sustained by high quality individuals. Amendments to this view highlight the role of T as immune-modulatory rather than -suppressive (Braude, Tang-Martinez, & Taylor, 1999; Da Silva, 1999; McDade, 2003), upregulating investment in secondary sex characteristics like the voice only in response to surplus immune and energetic capacity (Bribiescas, 2001; Ellison, 2001; Muehlenbein & Bribiescas, 2005). In this way, T—and the signal traits developmentally influenced by T—can serve as costly indicators of quality-dependent condition to potential mates and competitors.

Among adults (Dabbs & Malinger, 1999; Evans, Neave, Wakelin, & Hamilton, 2008; Hamdan et al., 2012; Puts, Apicella, & Cárdenas,

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2012) and adolescents (Harries, Walker, Williams, Hawkins, & Hughes, 1997; Pedersen, Moller, Krabbe, & Bennett, 1986), lower fundamental and formant frequencies are associated with higher T; however, no evidence currently links lower vocal frequencies with any indicators of phenotypic quality such as health or physical condition. Demonstration of relationships between circulating T, phenotypic quality, and vocal masculinization has been conspicuously missing, especially given the large evolutionary literature on vocal attractiveness and dominance judgments (e.g. Collins, 2000; Feinberg et al., 2005, 2006; Hodges-Simeon et al., 2010; Hodges-Simeon, Gaulin, & Puts, 2011; Puts et al., 2006, 2007; Wolff & Puts, 2010).

To explore these relationships, the present study assessed the degree to which vocal masculinization is influenced by immunological and energetic condition during adolescence, and evaluated the role of T as a possible mediator of this hypothesized developmental relationship. We target adolescence because sexual differentiation and, ultimately, final adult dimensions of the vocal tract develop during this time. Adolescence is also an important period for negotiation of status hierarchies (Ellis, Shirtcliff, Boyce, Deardorff, & Essex, 2011), especially in small-scale societies where individuals often remain with the same peers through life. We predict that those in better energetic and immune condition will have lower fundamental and formant frequencies after controlling for age in our cross-sectional sample. With structural equation modeling we also test whether T mediates the relationship between condition and masculine voice characteristics. Among adults, energetic deficits (Friedl et al., 2000; Trumble, Brindle, Kupsik, & O'Connor, 2010) and immunological load (Simmons & Roney, 2009) are associated with lower T levels, and lower T levels impact vocal frequencies (Dabbs & Malinger, 1999; Evans et al., 2008; Hamdan et al., 2012). Chronic energy shortages due to high workloads, high pathogen load or other insults during the period of vocal tract development may result in less masculine age-adjusted adolescent (and, ultimately, adult) voices (Hodges-Simeon, Gurven, Cardenas, & Gaulin, 2013). Evidence of these relationships between stressors, hormone levels and voice parameters during the canalization period for sexual differentiation of the vocal tract would provide support for the costly-signaling model of male voice.

In this study vocal masculinization is indexed using three vocal parameters associated with greater dominance and attractiveness ratings in previous studies: low fundamental frequency (F_0; Feinberg et al., 2006; Puts et al., 2007), lower and more closely spaced formants (i.e., *formant position*, P_f; Puts et al., 2012), and lower F_0 variation (F_0-SD; Hodges-Simeon et al., 2010, 2011; Puts et al., 2012). F_0 is the primary determinant of perceived pitch, and formants provide a resonant quality or timbre to a voice.

We assess condition in two ways: energetic availability and immune function. Energetic budget is assessed using measures of adiposity; individuals with greater fat stores have greater available energy to allocate to biological demands. Immune functioning is measured using a biomarker of adaptive mucosal immunity, secretory IgA (sIgA). sIgA is the dominant immunoglobulin on mucosal surfaces (e.g., saliva, tears, breast milk), acting as the initial defence against invading pathogens in the oral and nasal cavities, respiratory system, gastrointenstinal tract, and genito-urinary tract (Brandtzaeg, 2009). Low sIgA is indicative of depressed immunity and is associated with an increased risk of infections, particularly those of the upper respiratory tract (Drummond & Hewson-Bower, 1997; Fahlman & Engels, 2005; Nakamura, Akimoto, Suzuki, & Kono, 2006).

2. Methods

2.1. Population

The Tsimane are a small-scale, kin-based society of forager–horticulturalists in the lowland Amazonian forests of central Bolivia (Godoy et al., 2006; Gurven, Kaplan, & Supa, 2007; Gurven, Kaplan, Winking, Finch, & Crimmins, 2008). The Tsimane obtain the majority of calories from non-market sources (Martin et al., 2012), and experience more energetically demanding conditions than in the developed world, including calorically demanding workloads, limited food supply and medical access, and no sanitation or water treatment infrastructure. These factors contribute to an immunologically challenging environment, one characterized by high rates of parasite infection (Vasunilashorn et al., 2010), respiratory and gastrointestinal disease (Gurven et al., 2008), and anemia (Vasunilashorn et al., 2010). Measures of phenotypic quality were specifically selected for this energetic- and immune-challenged population. Our measures of fat stores (Tsimane-specific BMI-for-age and body fat percentage) capture variation in adipose stores relative to peers, reflecting the sum of energetic (including immunological) demands to the phenotype. The high rates of periodontal and respiratory infections in the Tsimane in particular are targeted using sIgA levels.

2.2. Participants

Ninety-one males, aged 8 to 22 ($M \pm SD = 13.6 \pm 3.3$), participated in the present study. Ages were estimated by integrating multiple sources of information. Participants were asked their age and date of birth during testing sessions. Then, stated age was checked against the Tsimane Health and Life History Project (THLHP) census. Censuses have been collected and updated annually since 2002, in conjunction with demographic interviews on most adults. The demographic interviews employ a combination of methods (e.g., relative age list anchored by people of known age, photo comparisons with individuals of known ages, reconciling multiple sources of information on several generations), for aging adults and children (Gurven et al., 2007). Census ages are most accurate for children and adolescents because parents were interviewed when their child was young. Parents of the current participants are also more likely to keep documentation of their child's birth than earlier generations. For these reasons, census age was used when in conflict with stated age or when participants were unsure of their exact date of birth. Eight participants who did not know their birth date also could not be found in the census. These individuals were assigned their stated whole number age plus 0.5. Omitting these eight did not change the reported results, and therefore they are included in the sample.

2.3. Anthropometrics

Height and weight were measured in accordance with standard protocols (Lohman, Roche, & Martorell, 1988). To provide data on relative energetic status, age-standardized residuals were calculated for body mass index using Tsimane-specific BMI-for-age curves (BMI-R; Blackwell, Snodgrass, Madimenos, & Sugiyama, 2010). A second measure of energetic status—body fat percentage—was used in parallel with BMI-R in all analyses. This second measure uses the Slaughter algorithm, which is designed to accurately calculate body-fat percentage for adolescents using tricep and subscapular skinfolds (Slaughter et al., 1988).

2.4. Saliva collection and analysis

Participants discharged 1 mL of relatively bubble-free saliva via passive drool into a polystyrene cryotube. In order to mitigate contamination, participants rinsed their mouths with clean water prior to saliva collection. Because secretory IgA is affected by saliva flow rate, (Kugler, Hess, & Haake, 1992; Miletic, Schiffman, Miletic, & Sattely-Miller, 1996), start and end times of saliva collection were recorded and secretion rate was multiplied by the total concentration. Therefore, sIgA is expressed as a flow rate in μg/min. Cryotubes were then stored in liquid nitrogen while in Bolivia and transported on dry ice to University of California Santa Barbara where they remained frozen (at −80 °C) until analysis, at which time they were shipped on dry ice to Salimetrics LLC (State College, PA).

Table 1
Correlations, means, and standard deviations for all study variables.

	BMI-R	Fat %	sIgA	Age	T	F_0	P_f	F_0-SD
BMI-R	–		0.02	–	0.45***	−0.23*	−0.36**	0.09
Fat %	0.57***	–	0.12	–	0.34**	−0.23*	−.11	−0.08
sIgA	0.03	0.19†	––	–	0.18	−0.09	−0.04	−0.12
Age	0.10	0.33**	0.31**	–	–	–	–	–
T	0.28**	0.41***	0.35***	0.82***	–	−0.38**	−0.41***	−0.05
F_0	−0.17	−.34**	−0.29**	−0.78***	−0.78***	–	0.47***	0.36**
P_f	−0.27*	−0.29**	−0.27*	−0.80***	−0.80***	−0.80***	–	0.14
F_0-SD	0.11	−0.12	−0.16	−0.16	−0.15	0.34**	−0.20†	–
Mean	0.01	14.86	24.92	13.70	50.18	197.36	1.09	68.97
SD	1.61	3.47	21.61	3.43	38.06	55.33	1.16	39.69

Note. BMI-R: body mass index age-standardized residual; fat %: body-fat percentage; sIgA: secretory IgA secretion rate (μg/min); T: testosterone (pg/mL); F_0: fundamental frequency (Hz); P_f: formant position; F_0-SD: variation in fundamental frequency (Hz).
The upper triangle shows partial correlations controlling for age.
Means and SDs are from non-transformed variables.
† $p < 0.10$.
* $p < 0.05$.
** $p < 0.01$.
*** $p < 0.001$.

T exhibits a diurnal rhythm in adult males; levels are higher in the morning than the evening. In the current study, testing sessions were scheduled to accommodate participants' school and family obligations. The average time of day for testing was 1:22 PM (SD = 169 minutes, range = 8:39 AM to 6:09 PM); however, times clustered in morning (M = 10:27, N = 67) and afternoon sessions (M = 15:35, N = 88). T and time of day were not significantly associated (linearly or non-linearly) in our sample, and time of day did not explain any additional variance when it was included in analyses (see Results). Several reasons may explain this lack of correlation. First, approximately 85% of participants contributed saliva four or more hours after waking (and 100% after two hours), when T would be leveling off for those with an established diurnal pattern (Butler et al., 1989). Second, higher AM T levels have been documented for older adolescents and adults only. Salivary testosterone levels of well-nourished boys do not initiate diurnal rhythms until at least Tanner genital stage 3 (Butler et al., 1989), after which development continues into adulthood. In the current study, there was no significant difference in T between morning and afternoon sessions, even after boys were grouped into developmental stages (pre-, peri-, and post-pubertal). It is possible that the establishment of full diurnal T patterns may occur later in non-industrialized, developmentally delayed populations like the Tsimane. Among the Ache—an energetically stressed population in Paraguay—peak AM:PM T ratio did not occur until the third decade (Bribiescas & Hill, 2010). Finally, age-related change in T is substantial in this age group, such that it captures most of the inter-individual variation in T levels. No studies have examined the development of diurnal T rhythms in adolescence in an energetically stressed population; this is an important area for future research.

All assays were performed in duplicate using a sensitive competitive enzyme immunoassay (EIA) protocol (Salimetrics LLC). Analytes are measured in picograms per milliliter (pg/mL; T) or micrograms per milliliter (μg/mL; sIgA). Average intra-assay and inter-assay coefficients of variation were as follows: T (4.6% and 9.8%) and sIgA (5.6% and 8.8%). In all analyses, "sIgA" refers to sIgA secretion rate in μg/min.

Because serum T is higher than salivary T, transferrin (an iron-binding glycoprotein found in blood) was assayed using EIA (Salimetrics LLC; M ± SD = 0.91 ± 0.89, range: 0.08–5.0) in order to adjust for potential blood contamination (Kivlighan et al., 2004). Two participants with transferrin levels greater than three standard deviations above the mean were eliminated.

2.5. Acoustic recording and analysis

Participants were shown five photographs representing easily recognized objects in Tsimane life and asked to name each object. The particular words were chosen based on the presence of terminal vowel sounds (*míshi*: "ee", *açhuj*: "oo", *pe're*: "ā", *perota*: "ah", and *ococo*: "oh"). Voice samples were recorded with an Audio-Technica lavalier microphone using a Sony PCM-M10 digital audio recorder. An adjustable headset was used to standardize the microphone's distance from the lips (5 cm) and to reduce background noise. Samples were recorded in mono using a sampling rate of 44.1 kHz and 16-bit quantization. Recordings were saved as high-quality uncompressed linear PCM.wav files.

For all participants' recordings, mean F_0 (and standard deviation; F_0-SD) and formant structure (P_f), all measured in Hertz (Hz), were determined using Praat voice analysis software (Version 5.1.37; Boersma & Weenick, 2010). Formants were ascertained using methods and scripts used by Puts et al. (2012), although formant ceilings were adjusted separately for different developmental groups defined by the Pubertal Development Scale (PDS; Petersen, Crockett, Richards, & Boxer, 1988). The PDS is a self-report pubertal scale that has been shown to be a reliable approximation of the Tanner stages (Brooks-Gunn, Warren, Rosso, & Gargiulo, 1987; Coleman & Coleman, 2002). Males in stages 1 and 2 (mean age = 10.9 ± 1.6) were measured using a formant ceiling of 7000 Hz. Males in stage 3 (mean age = 15.0 ± 1.5) were measured using a formant ceiling of 6000 Hz. And males in stages 4 and 5 (mean age = 18.2 ± 2.3) were measured with a formant ceiling of 5000 Hz. F_1 through F_4 were measured at each glottal pulse, targeting voiced speech only, and then averaged for analyses (mean number of glottal pulses per file = 527 ± 273). In order to calculate P_f, the first four formants were standardized using means and standard deviations (Puts et al., 2012).

2.6. Data analysis

The following variables were log transformed to correct for non-normality: T, sIgA, age, F_0, P_f, and F_0-SD. Path analyses and data imputation were conducted using Amos software (version 21.0.0) and maximum likelihood estimation. Twelve participants had at least one missing value, due to either obligations that cut the session short or to a malfunctioning liquid nitrogen tank. With a 7:1 ratio of participants-to-parameters, sample size was adequate. Model fit was assessed with a joint consideration of three statistics routinely used in structural equation modeling: 1) the chi-square statistic (χ^2), which compares the fit between the covariance matrix for the observed data with that of the specified theoretical model; 2) the Comparative Fit Index (CFI), which addresses the discrepancies between the hypothesized model and the sample data, adjusting for sample size; and 3) the root mean square error of approximation (RMSEA), an absolute (versus comparative) measure of fit. Good model fit is evidenced by a non-significant chi-square, a CFI of at least 0.95, and an RMSEA of 0.05 or less (Byrne, 2001).

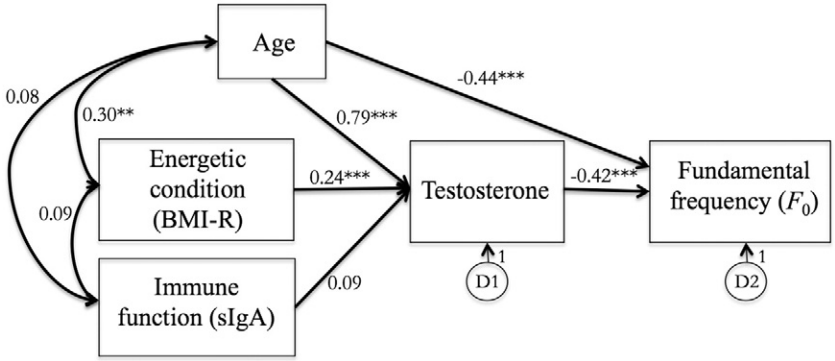

Fig. 1. Standardized parameter estimates for the hypothesized model predicting F_0 (model A).

3. Results

3.1. Descriptive statistics and correlations among variables

Correlations, means, and standard deviations are shown in Table 1. As a first step, the association between T and sIgA was examined as a test of T's immunomodulatory action. T was significantly correlated with sIgA and age. That is, individuals with higher T had higher levels of mucosal immunity. Controlling for age, T and sIgA were positively associated, however the correlation did not achieve significance. T also appeared to be sensitive to energetic condition; males with higher BMI-R and higher body fat percentage had higher T levels. This association was stronger after controlling for age. See Table 1.

As a second step, we examined the associations between phenotypic condition and masculine vocal parameters. Energetic condition (BMI-R and body fat percentage) and sIgA were uncorrelated, and therefore represent independent measures of phenotypic condition. Age and energetic condition (controlling for age) were inversely associated with F_0 and P_f. Secretory IgA was significantly negatively correlated with F_0 and P_f, however this association did not hold after controlling for age.

3.2. Model specification

The following analyses explore the possible theoretical pathway through which phenotypic condition may affect differential development of masculine voice characteristics in adolescence. The model proposes that those who are in better condition (i.e., those with higher energetic reserves and better immune functioning) can afford higher levels of T, and that this higher T will lead to more "masculine" voices (i.e., lower F_0, more narrow P_f, and less variable F_0-SD).

3.3. Model testing

We assessed models predicting F_0, P_f, and F_0-SD separately. Models are specified A, B, C, D, E, or F depending on the structure of pathways and parameters, not on the prediction of a specific vocal parameter.

Table 2
Summary of model fit (see note for model descriptions).

	χ^2	$\Delta\chi^2$	CFI	RMSEA (90% CI)
Predicting F_0				
A	0.594, $p > .05$	–	1.000	0.000 (0.000–0.145)
B	0.050, $p > .05$	0.444, *ns*[a]	1.000	0.000 (0.000–0.169)
C	0.055, $p > .05$	0.045, *ns*[a]	1.000	0.000 (0.000–0.251)
D	0.607, $p > .05$	–	1.000	0.000 (0.000–0.079)
E	0.063, $p > .05$	0.544, *ns*[b]	1.000	0.000 (0.000–0.000)
F	0.549, $p > .05$	0.063, *ns*[b]	1.000	0.000 (0.000–0.251)
Predicting P_f (using body-fat percentage for energetic status)				
A	0.159, $p > .05$	–	1.000	0.000 (0.000–0.070)
B	0.092, $p > .05$	0.067, *ns*[a]	1.000	0.000 (0.000–0.360)
C	0.077, $p > .05$	0.082, *ns*[a]	1.000	0.000 (0.000–0.183)
D	0.393, $p > .05$	–	1.000	0.000 (0.000–0.035)
E	0.335, $p > .05$	0.058, *ns*[b]	1.000	0.000 (0.000–0.117)
F	0.295, $p > .05$	0.098, *ns*[b]	1.000	0.000 (0.000–0.110)
Predicting P_f (using BMI-for-age residuals for energetic status)				
A	6.987, $p < .05$	–	0.975	0.166 (0.044–0.308)
B	0.086, $p > .05$	6.901, $p < .05$[a]	1.000	0.000 (0.103–0.450)
C	6.883, $p < .05$	0.008, *ns*[a]	0.971	0.256 (0.000–0.242)
D	7.000, $p < .05$	–	0.980	0.122 (0.000–0.242)
E	0.099, $p > .05$	6.901, $p < .05$[b]	1.000	0.000 (0.000–0.000)
F	6.901, $p < .05$	0.099, *ns*[b]	0.976	0.165 (0.042–0.307)
Predicting F_0-SD				
A	2.196, $p > .05$	–	0.998	0.033 (0.000–0.214)
B	0.851, $p > .05$	1.345, *ns*[a]	1.000	0.000 (0.000–0.270)
C	1.322, $p > .05$	0.874, *ns*[a]	0.997	0.060 (0.000–0.295)
D	2.209, $p > .05$	–	1.000	0.000 (0.000–0.158)
E	0.864, $p > .05$	1.345, *ns*[b]	1.000	0.000 (0.000–0.163)
F	1.344, $p > .05$	0.864, *ns*[b]	1.000	0.000 (0.000–0.186)

Note. For model A, see Fig. 1. Model B builds on model A by adding a direct path from BMI to the vocal parameter. Model C adds a direct path from sIgA to the vocal parameter. For model D, see Fig. 1. Model E builds on model D by adding a direct path from BMI to the vocal parameter. Model F adds a direct path from sIgA to the vocal parameter.

[a] $\Delta\chi^2$ from model A.

[b] $\Delta\chi^2$ from model D.

Table 3
Parameter estimates and critical ratios for the final (best fitting) models predicting F_0, P_f, and F_0-SD.

Parameter	Unstandardized estimate	Standard error	Critical ratio (z)	p-value	Standardized estimate
Variances					
BMI-R	0.007	0.001	6.708	<.001	
Body-fat %	11.880	1.800	6.600	<.001	
sIgA	0.094	0.015	6.215	<.001	
Age	0.012	0.002	6.708	<.001	
D1	0.026	0.004	6.324	<.001	
D2	0.006	0.001	6.311	<.001	
Covariances					
BMI-R, sIgA	0.002	0.003	0.745	.456	
BMI-R, Age	0.001	0.001	0.713	.476	
sIgA, Age	0.010	0.004	2.540	.011	
Body-fat %, sIgA	0.241	0.123	1.966	.049	
Body-fat %, Age	0.124	0.042	2.985	.003	
Path coefficients: Predicting F_0 (model A; see Fig. 1)					
BMI-R➔T	0.968	0.220	4.393	<.001	0.244
sIgA➔T	0.091	0.063	1.446	.148	0.085
Age➔T	2.390	0.176	13.543	<.001	0.785
Age➔ F_0	−0.542	0.141	−3.849	<.001	−0.441
T➔ F_0	−0.169	0.046	−3.651	<.001	−0.419
Path coefficients: Predicting P_f (model A)					
Body-fat %➔T	0.020	0.006	3.294	<.001	0.206
sIgA➔T	0.087	0.067	1.303	.193	0.082
Age➔T	2.229	0.195	11.445	<.001	0.731
Age➔ F_0	−0.366	0.090	−4.085	<.001	−0.440
T➔ F_0	−0.120	0.029	−4.079	<.001	−0.440
Path coefficients: Predicting P_f (model E; see Fig. 3)					
T➔ BMI-R	0.198	0.044	4.489	<.001	0.788
T➔ sIgA	0.280	0.180	1.556	0.120	0.298
Age➔ T	2.390	0.176	13.543	<.001	0.785
BMI-R➔ P_f	−0.189	0.071	−2.671	<.01	−0.173
Age➔ P_f	−0.366	0.091	−4.041	<.001	−0.441
T➔ P_f	−0.119	0.030	−4.003	<.001	−0.438
Path coefficients: Predicting F_0-SD (model A)					
BMI-R➔T	0.968	0.220	4.393	<.001	0.244
sIgA➔T	0.091	0.063	1.446	.148	0.085
Age➔T	2.390	0.176	13.543	<.001	0.785
Age➔ F_0-SD	−0.374	0.639	−0.586	.558	−0.116
T➔ F_0-SD	−0.054	0.210	−0.259	.796	−0.051

Note. BMI-R: body mass index residual; body-fat %: body fat percentage; sIgA: secretory IgA secretion rate; T: testosterone; F_0: fundamental frequency; P_f: formant position; F_0-SD: variation in fundamental frequency.
Model fit summary: Predicting F_0: $\chi^2(2, N = 91) = 0.594$, *ns*; CFI = 1.00; RMSEA = 0.000 (90% CI = .000–.145). Predicting P_f (model A): $\chi^2(2, N = 91) = 0.159$, *ns*; CFI = 1.00; RMSEA = 0.000 (90% CI = .000–.070). Predicting P_f (model E): $\chi^2(2, N = 91) = 0.335$, *ns*; CFI = 1.000; RMSEA = 0.000 (90% CI = .000–.117). Predicting F_0-SD: $\chi^2(2, N = 91) = 2.196$, *ns*; CFI = 0.998; RMSEA = .033 (90% CI = .000–.214).

For example, three separate model A's are described below, one for each vocal parameter (see Fig. 1 for model A predicting F_0). Immune condition was indexed by sIgA. Age was included as a control, with paths to T and the voice parameters. Energetic condition was indexed in two ways: BMI-for-age standardized residuals (BMI-R) calculated using Tsimane-specific growth curves, and body-fat percentage based on skinfold data. For models predicting F_0 and F_0-SD, these two condition variables yielded nearly identical parameter estimates and measures of model fit. Therefore, we only present those models using BMI-R. However, for models predicting P_f, body-fat percentage produced stronger results for a T-mediated costly signaling model. We therefore present results for models predicting P_f using both BMI-R and body-fat percentage.

3.3.1. Models predicting F_0

Model A assumes that T fully mediates the relationship between energetic and immune condition effects on vocal parameters. Predicting F_0, model A was a good fit (see Fig. 1 for standardized beta values in model A, Table 2 for model fit comparisons, and Table 3 for parameter values and critical ratios). However, it is possible that the effects of BMI-R and sIgA on F_0 are only partially mediated by T. Therefore, we tested two nested models in which we added direct effects between BMI-R and F_0 (model B), and between sIgA and F_0 (model C), one at a time, and computed the change in the chi-square statistic ($\Delta\chi^2$). A significant $\Delta\chi^2$ indicates that the addition of the direct effect significantly improved model fit. Neither direct paths from BMI-R to F_0 in model B nor ($\Delta\chi^2 = 0.444$, *ns*) from sIgA to F_0 in model C improved model fit ($\Delta\chi^2 = 0.045$, *ns*). Thus, we conclude that the initial, fully mediated model provides a better fit to the data than the partially mediated models, suggesting that males in better energetic condition have lower voices because of the effects of T. These results are consistent with our proposed theoretical model. They suggest that individuals in better condition (as reflected by higher BMI-for-age relative to Tsimane norms) had higher T levels, and that those with higher T levels had lower, more masculine F_0. Further, the relationship between condition and F_0 appeared to be mediated by T levels. Table 2 provides a summary of fit statistics for all models.

Next, to rule out the possibility that both energetic and immune condition are sensitive to changes in testosterone rather than the other way around, we tested an alternative model (model D). Variables were reordered so that testosterone predicted BMI, sIgA, and F_0. Age was again used as a control, with paths to testosterone, BMI, sIgA, and F_0 (see Fig. 2). Because this alternative model and our original model were not nested, we were unable to compute a chi-square difference test between them. However, the fit indices for this alternative model revealed a similarly good fit to the data as the original model A, with greater *df* [$\chi^2(3, N = 91) = 0.607$, $p > .05$; CFI = 1.00; RMSEA = .00 (90% CI = .00–.079)]. A nested model (model E) was then performed with a direct path from BMI-R to F_0. Fit statistics for this model were not substantially better than model D [$\Delta\chi^2 = 0.544$, *ns*; $\chi^2(2, N =$

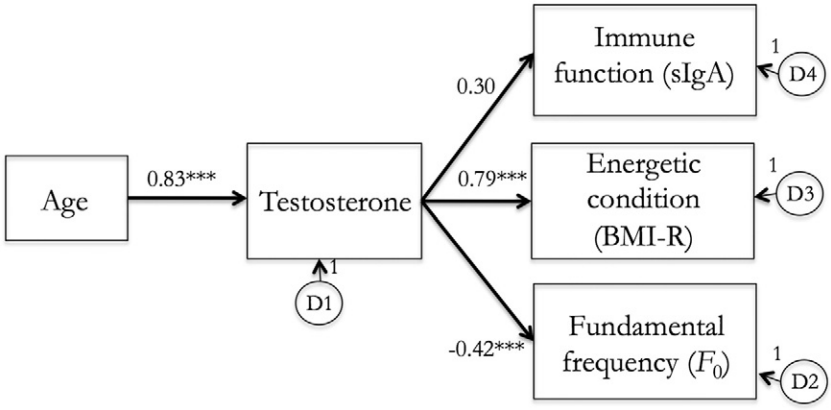

Fig. 2. Standardized parameter estimates for the alternative model predicting F_0 (model D).

91) = 0.063, p > 0.05; CFI = 1.000; RMSEA = 0.000 (90% CI = .000–.000)]. Finally, a second nested model was performed with a path from sIgA to F_0 [model F; $\Delta\chi^2 = 0.063$, *ns*; $\chi^2(2, N = 91) = 0.549$, p > .05; CFI = 1.000; RMSEA = 0.000 (90% CI = .000–.251)]. Again, model D was not improved upon. Model D (Fig. 2), however, performed as well as model A, and therefore presented an equally plausible scenario, both statistically and theoretically.

3.3.2. Predicting P_f

3.3.2.1. Predicting P_f using BMI-R as a measure of energetic status. A similarly structured model A, predicting P_f, did not show an adequate fit to the data. Including a direct path from BMI-R to P_f significantly improved model fit statistics; (model B; $\Delta\chi^2 = 6.901$, p < .05); however, the addition of a path from sIgA to P_f did not (model C; $\Delta\chi^2 = 0.008$, *ns*).

We also tested the alternative model predicting P_f, model D. Variables were reordered so that testosterone predicted BMI-R, sIgA, and P_f—with age as a control. Fit statistics were slightly better than model A or B, however the fit was still inadequate [$\chi^2(3, N = 91) = 7.000$, p > .05; CFI = 0.980; RMSEA = 0.122 (90% CI = .000–.242)]. We then added a direct pathway from BMI-R to P_f (model E). Addition of this pathway significantly improved fit statistics from model D ($\Delta\chi^2 = 6.901$, p < .05) and fit the data well [$\chi^2(2, N = 91) = 0.099$, p > .05; CFI = 1.000; RMSEA = 0.000 (90% CI = .000–.000)]. Model F was not a good fit to the data [$\Delta\chi^2 = 0.099$, *ns*; $\chi^2(2, N = 91) = 6.901$, p < .05; CFI = 0.976; RMSEA = 0.165 (90% CI = .042–.307)]. Model E, shown in Fig. 3, describes a scenario where T increases as a product of development, with immune function, energetic reserves, and voice development following as a consequence. In both models (A and E), however, P_f is significantly predicted by both T and energetic condition.

3.3.2.2. Predicting P_f using body-fat percentage as a measure of energetic status. Substituting a second measure of energetic status—body-fat percentage—in model A, predicting P_f, produced a good fit to the data [$\chi^2(2, N = 91) = 0.159$, p > .05; CFI = 1.000; RMSEA = 0.000 (90% CI = .000–.070)]. See Table 2 for summaries of model fit. Including direct paths from body-fat percentage to P_f (model B; $\Delta\chi^2 = .067$, *ns*) and from sIgA to P_f did not improve fit statistics (model C; $\Delta\chi^2 = 0.082$, *ns*). We also tested the alternative model predicting P_f, model D. Fit statistics were virtually unchanged from model A [$\chi^2(3, N = 91) = 0.393$, p > .05; CFI = 1.000; RMSEA = 0.000 (90% CI = .000–.035)]. Neither model E ($\Delta\chi^2 = 0.058$, *ns*) nor model F improved upon model D ($\Delta\chi^2 = 0.098$, *ns*).

3.3.3. Predicting F_0-SD

Predicting F_0-SD, model A provides a reasonable fit [$\chi^2(2, N = 91) = 2.196$, p > .05]. Neither model B [$\Delta\chi^2 = 1.345$, *ns*; $\chi^2(1, N = 91)$] nor model C [$\Delta\chi^2 = 0.874$, *ns*; $\chi^2(1, N = 91) = 1.322$, p > .05] improved upon model A. Although the models provide an adequate fit to the data, Beta values for paths from age to F_0-SD, T to F_0-SD, BMI-R to F_0-SD, and sIgA to F_0-SD were not statistically significant. Therefore, our prediction was not confirmed for F_0-SD. See Tables 2 and 3.

The alternative model D also performed well, with slightly better fit statistics than model A [$\chi^2(3, N = 91) = 2.209$, p > .05; CFI = 1.000; RMSEA = 0.000 (90% CI = .000–.158)]. Neither model E [$\Delta\chi^2 = 1.345$, *ns*; $\chi^2(2, N = 91) = 0.864$, p > .05; CFI = 1.000; RMSEA = 0.000 (90% CI = .000–.163)] nor model F [$\Delta\chi^2 = 0.864$, *ns*; $\chi^2(2, N = 91) = 1.344$, p > .05; CFI = 1.000; RMSEA = 0.000 (90% CI = .000–.186)] provided a better fit than model D.

4. Discussion

According to a costly-signaling model of male voices (Folstad & Karter, 1992; Hamilton & Zuk, 1982; Muehlenbein & Bribiescas, 2005; Zahavi, 1975), masculine voice characteristics reveal fitness-relevant information about a speaker's quality or condition to potential mates and competitors. In the present study, we find evidence that masculine voice characteristics are costly signals of quality. First, results show an association between phenotypic condition (as measured by high BMI age-standardized residuals, body-fat percentage, and sIgA levels) and masculine male voice characteristics (Hamilton & Zuk, 1982), and between phenotypic condition (as measured by BMI-R and body-fat percentage) and T. The association between T and mucosal immunity, although not significant after controlling for age, trended in a positive direction. Second, we find a relationship between vocal masculinity and T (Folstad & Karter, 1992). Finally, we evaluated T as a mediator between phenotypic condition and voice (Muehlenbein & Bribiescas, 2005). For two defining features of men's voices (low mean F_0 and lower, narrower P_f), we found support for our proposed theoretical model. T mediated the association between phenotypic condition and low F_0 and P_f in our sample of adolescent Tsimane males.

This is the first study to examine the relationship between condition and individual differences in adolescent male voice characteristics. A large literature on the evolutionary origins of sexually dimorphic male voices has invoked costly-signaling theories to explain the increased attractiveness and dominance attributed to males with masculine voice characteristics (e.g. Collins, 2000; Feinberg et al., 2005, 2006; Hodges-Simeon et al., 2010, 2011; Puts et al., 2006, 2007; Wolff & Puts, 2011). This literature rests on the assumption that voice parameters reveal fitness-relevant information; otherwise these features should be ignored, and their attractiveness and formidability would be unexplained.

Fitch and Giedd (1999) argue that sexual dimorphism in formant structure (including P_f) is better explained using an index model rather than a T-mediated costly signaling model. Index signals are those that vary simply as a function of body size rather than as a function of condition-dependent T levels. If such allometric constraints exist,

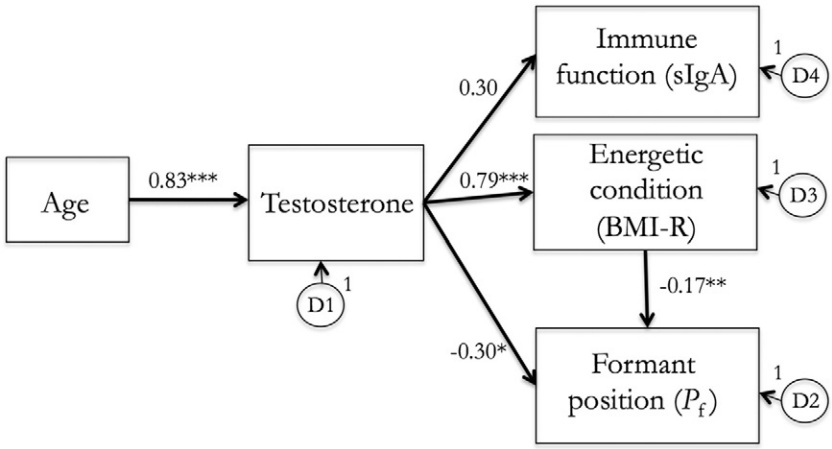

Fig. 3. Standardized parameter estimates for the alternative model predicting formant position (P_f; model E).

formant structure should scale with height throughout development and into adulthood. The empirical relationship between formants and height, however, remains unclear: Several studies have reported positive associations (González, 2004; Evans, Neave, & Wakelin, 2006; Puts et al., 2012), but others have not (Collins, 2000; Rendall, Kollias, & Ney, 2005; Sell et al., 2010). Further, an analysis of formant development during adolescence showed that it scaled more closely with strength than with height (Hodges-Simeon, Gurven, Puts, & Gaulin, 2014). These results—in conjunction with the zero-order correlations between P_f and T in the present sample—challenge the index model. In the present study, we found support for a T-mediated costly signaling model for P_f using body-fat percentage as a proxy energetic status.

Our mediational model (model A) was based on a theoretically informed hypothesis about the relationship between condition, T, and secondary sexual characteristics (Folstad & Karter, 1992; Hamilton & Zuk, 1982; Muehlenbein & Bribiescas, 2005; Zahavi, 1975). We also tested an alternative model (model D, see Fig. 2) in which T mediated the association between age and energetic status, sIgA, and voice characteristics. In other words, condition was specified as an outcome of T rather than an input. This is a theoretically plausible scenario. Evidence indicates that T decreases fat mass by upregulating adipose catabolism (Bhasin et al., 2005), suggesting that T both affects and is affected by the amount of adipose tissue. This latter model (D) provided a better fit to the data predicting P_f when BMI-R was used as the energy variable and was comparable in fit to the mediational model (A) predicting F_0. In both models (D and A), better energetic condition predicted lower F_0 and P_f; however, only in model A did T mediate this association.

In our sample of adolescent males, we found that individuals with greater energy reserves (BMI residuals and body-fat percentage) had lower F_0 and P_f. Similarly, Juul, Magnusdottir, Scheike, Prytz, and Skakkebaek (2007) found that 8-year-old boys with high BMI were more likely to have an earlier voice break. We also found that T mediated the relationship between energetic reserves and low F_0 and P_f. Individuals with greater energy reserves had higher T, even after controlling for age, and those with greater T had lower F_0 and P_f. These results accord with findings from previous studies demonstrating that both chronic (Ellison et al., 2002) and acute (Friedl et al., 2000; Trumble et al., 2010) deficits in energy balance are associated with lower T levels among adults.

Our measure of mucosal immunity, sIgA, was correlated with T, F_0, and P_f; however, these associations disappeared when age was controlled. Secretory IgA shows an age-related trend in the present sample, with sIgA increasing linearly throughout adolescence. Several studies have examined the ontogeny of sIgA, yet results have been inconsistent. For example, "adult" levels of sIgA have been reported at 12 months (Mellander, Carlsson, & Hanson, 1984), 7 years (Burgio, Lanzavecchia, Plebani, Jayakar, & Ugazio, 1980), 9 years (Gleeson et al., 1987), and 13 years (Ben-Aryeh, Fisher, Szargel, & Laufer, 1990). Two aspects of the disease ecology of the rural Bolivia may influence the later peak in sIgA among Tsimane adolescents compared with other samples. First, respiratory infections are common among the Tsimane. Two-thirds of early childhood deaths are due to infectious disease, of which respiratory infections account for half (Gurven et al., 2007). Lower sIgA has been associated with an increased incidence of upper respiratory infections (Drummond & Hewson-Bower, 1997; McClelland, Alexander, & Marks, 1982). Second, the Tsimane experience an extremely high rate of dental caries, which are associated with higher sIgA (Thaweboon, Thaweboon, Nakornchai, & Jitmaitree, 2008). Thus, Tsimane children may continue to upregulate sIgA throughout adolescence as an adaptive response to higher levels of respiratory and cariogenic pathogens. The facultative regulation of immune functioning as an adaptive response to environmental heterogeneity is an important area of current and future research in human ecological immunology (Blackwell et al., 2010; McDade, 2003).

All analyses in the present study controlled for age in order to bring developmental relationships between the variables to the forefront. In another analysis of these data, Hodges-Simeon et al. (2013) used growth models to show that a later age at peak voice velocity (i.e., the age at which vocal frequencies change most rapidly) and a faster rate of vocal change are associated with lower adult voices in US and well-nourished Tsimane boys compared with undernourished Tsimane boys. The timing, pace, and degree of development is often a manifestation of condition: Malnutrition, disease, high workload, and high altitude are all associated with delays in growth and reproductive maturation (for a review, see Bogin, 1999). In other words, individuals in poor condition develop later and slower than those in good condition. For aspects of the phenotype like height (Bogin, 1999) and voice (Hodges-Simeon et al., 2013), later and slower development leads to adult deficits (e.g. higher vocal frequencies). In this way, the adult acoustic phenotype acts as a record of adolescent condition.

We also found that our mediational model (A) predicting F_0-SD provided an adequate fit to the data; however, path coefficients were not significant, so the model was rejected. F_0-SD is sexually dimorphic in adults (Puts et al., 2012), and lower F_0-SD is associated with greater perceptions of physical dominance (Hodges-Simeon et al., 2010) and higher mating success (Hodges-Simeon et al., 2011) in US populations. F_0-SD may vary in response to changes in autonomic arousal, potentially making it a situational (as opposed to developmental) cue of relative dominance. If this is the case, we would not expect F_0-SD to vary with changes in condition or immune functioning during adolescence. In addition, the vocal clips were very short (i.e., five single words), which may not have generated adequate variation in F_0-SD. This is an important limitation of the study; future studies on the development of F_0-SD may benefit from the use of longer vocal clips.

Future research could expand upon the findings presented here in several ways. First, a larger sample of boys could facilitate analyses of the relationships between these variables at different points in adolescent development. Second, including additional biomarkers of immunity might provide an alternative picture of immune functioning. Finally, future studies should attempt a longitudinal investigation where the amount and rate of acquisition of fat stores in juvenility and early adolescence are assessed as antecedents to changes in T and masculine secondary sexual characteristics.

This is the first study to examine the relationships between masculine voice characteristics, T, and phenotypic condition in a preindustrial population experiencing energetic limitation and high disease risk, and to target these associations during the developmental stage when vocal tract dimensions, and hence voice characteristics, are canalized. Although speakers can manipulate their voice to some degree, stable individual differences exist in masculine vocal characteristics (Baken, 1987). Findings presented here suggest that this variation may be the result of differential vocal tract development in adolescence resulting ultimately from individual differences in energetic reserves and proximally from differences in T. As such, we provide support for a costly signaling model of masculine male voices.

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