Received: 3 March 2023 | Revised: 17 May 2023 | Accepted: 12 June 2023

DOI: 10.1002/ajhb.23949

ORIGINAL ARTICLE

American Journal of Human Biology WILEY

Energetic costs of testosterone in two subsistence populations

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Funding information
National Institutes of Health, Grant/Award Number: RF1AG054442; National Science Foundation, Grant/Award Number: 1748282

Abstract

Objective: Testosterone plays a role in mediating energetic trade-offs between growth, maintenance, and reproduction. Investments in a high testosterone phenotype trade-off against other functions, particularly survival-enhancing immune function and cellular repair; thus only individuals in good condition can maintain both a high testosterone phenotype and somatic maintenance. While these effects are observed in experimental manipulations, they are difficult to demonstrate in free-living animals, particularly in humans. We hypothesize that individuals with higher testosterone will have higher energetic expenditures than those with lower testosterone.

Methods: Total energetic expenditure (TEE) was quantified using doubly labeled water in $n = 40$ Tsimane forager-horticulturalists (50% male, 18–87 years) and $n = 11$ Hadza hunter-gatherers (100% male, 18–65 years), two populations living subsistence lifestyles, high levels of physical activity, and high infectious burden. Urinary testosterone, TEE, body composition, and

physical activity were measured to assess potential physical and behavioral costs associated with a high testosterone phenotype.

Results: Endogenous male testosterone was significantly associated with energetic expenditure, controlling for fat free mass; a one standard deviation increase in testosterone is associated with the expenditure of an additional 96–240 calories per day.

Discussion: These results suggest that a high testosterone phenotype, while beneficial for male reproduction, is also energetically expensive and likely only possible to maintain in healthy males in robust condition.

1 | INTRODUCTION

Steroid hormones play a critical role in modulating energy use, affecting and affected by numerous fitness-relevant domains including behavior, immune activation, and reproductive physiology. While many hormones modulate caloric demand and utilization, testosterone is particularly important as it influences classic life history trade-offs between energetic investments in growth (e.g., muscle formation), reproduction, and the maintenance of immune function (Bhasin et al., 1996; Folstad & Karter, 1992; Griggs et al., 1989; Jasienska et al., 2017; Muehlenbein & Bribiescas, 2005; Sinha-Hikim et al., 2003). Most testosterone research motivated by life history theory focuses on males, as there are more direct fitness payoffs and tradeoffs in relation to male testosterone and reproductive behavior (Folstad & Karter, 1992; Muehlenbein & Bribiescas, 2005), whereas in vertebrate females, links between energetics and reproduction are not as dependent on testosterone. However, energetic trade-offs and costs and benefits of testosterone extend to females, as immune activation and musculature can have significant fitness impacts for both sexes across diverse species and contexts (Gurven, Trumble, et al., 2016; Iannuzzi-Sucich et al., 2002; Kraft et al., 2021).

While life history theory provides a general framework for understanding why a high testosterone phenotype may be energetically costly in terms of immune activation (Jasienska et al., 2017; Muehlenbein & Bribiescas, 2005), secondary sexual signals (Folstad & Karter, 1992), or the cost of building and maintaining muscle tissue (Bribiescas, 2010), few empirical studies have quantified these costs, particularly for humans as measuring energetic expenditure in free-living conditions is extremely difficult (Kraft et al., 2021; Leonard, 2012). The potential energetic costs of a high testosterone phenotype are particularly difficult to assess in real world conditions because strenuous physical activity results in both acute increases in testosterone and energetic expenditure (Jensen et al., 1991; Trumble et al., 2013, 2014). It is critical to not only measure physical activity, but also a comprehensive measure of energetic expenditure, such as total energetic expenditure (TEE; kcal/d) (Jensen et al., 1991; Trumble et al., 2013, 2014). Doubly labeled water (DLW) has emerged as a well-validated and minimally invasive method for measuring the TEE of free-living organisms, although due to logistics and expense, DLW has rarely been used in non-industrialized populations (Kraft et al., 2021; Pontzer et al., 2012, 2015), and then only with relatively small samples.

Experiments in male house sparrows (*Passer domesticus*) indicate that testosterone increases both basal metabolic rate and secondary sexual signals like plumage (Buchanan et al., 2001). In other avian models (e.g., *Junco hyemalis*) and mountain spiny lizards (*Sceloperous jarrovi*), exogenous male testosterone increases physical activity (Lynn et al., 2000; Marler et al., 1995). Unfortunately, the caloric cost of an endogenously high testosterone phenotype remains poorly understood, as experimental studies differ widely in the duration, timing of delivery, and the level of exogenous testosterone delivered (normal range vs. supraphysiological doses). Most associations between testosterone and energetic status in humans to date have focused on testosterone declines following energetic stressors, including minor immune activation from an influenza vaccination (Simmons & Roney, 2009), injury (Spratt et al., 2008), and decreased caloric intake (Cameron, 1996; Trumble et al., 2010). The greater the insult, the larger the subsequent decline in testosterone.

Contemporary subsistence populations living traditional lifestyles offer insights into environmental challenges faced throughout human history, though extant populations vary widely in geography, culture and lifestyle. While no single subsistence-level socio-ecology represents the diverse array of environments experienced by our hominin ancestors, populations like the Hadza hunter-gatherers of Tanzania, and the Tsimane forager-horticulturalists of Bolivia represent critical case studies of the energetic costs of testosterone, as both populations

forage, hunt, or grow the calories they consume, in remote, high pathogen and high fertility contexts (Kraft et al., 2021). Men from diverse subsistence populations with lower energetic surplus and higher rates of immune activation have significantly lower levels of testosterone at younger ages compared to men in industrialized populations (Ellison et al., 2002; Trumble et al., 2012), suggesting that in environments where total energy budgets are limited, scarce resources are invested in immune function and physical activity instead of a high testosterone phenotype (Bribiescas, 2010; Muehlenbein & Bribiescas, 2005). The Tsimane of neotropical Bolivia, one of the subsistence populations examined here, generally have higher pathogen burden (Blackwell et al., 2016), physical activity levels (Gurven et al., 2013), and fertility than industrialized populations (Blackwell et al., 2015; Costa et al., 2018), which together can result in lower energy balance. Similarly, Hadza hunter-gatherers living in the savannah-woodlands of northern Tanzania practice a traditional lifeway similar to those experienced throughout much of our human evolutionary past (Pontzer et al., 2012; Raichlen et al., 2017), with relatively high levels of physical activity (Pontzer et al., 2012).

Here we employ a cross-sectional sample of Tsimane men and women, and Hadza men to assess the energetic costs a high testosterone phenotype. We assess associations between TEE and urinary testosterone, while adjusting for age, physical activity, and body composition. We test the hypothesis that, under conditions of limited energy availability, testosterone is a metabolically costly investment, particularly for men. Thus higher testosterone should be associated with greater energetic expenditure. A secondary prediction is that the effect of testosterone on energy expenditure will be greater among men than women. Because physical activity is known to influence testosterone, we controlled for physical activity levels, measured using accelerometry, to determine whether testosterone and TEE were associated independently of activity effects.

2 | MATERIALS AND METHODS

2.1 | Tsimane data collection

Forty adult Tsimane participants (50% male) were administered (day 0) one oral DLW dose (114 g for men, 79 g for women; 10% $H_2{}^{18}O$, 6% ^2H_2O) in August 2013 (Gurven, Trumble, et al., 2016; Pontzer et al., 2012, 2015). First morning void urine specimens were collected, on the morning following their dose (day 1), and thereafter on days 3, 5, 7, 8, and 9. Seven participants failed to provide urine specimens on day 8, and 27 participants failed to provide specimens on day 9. Specimens were collected in plastic receptacles and transferred to 2 mL cryovials, which were then stored in a liquid nitrogen tank for up to 2 months before transfer on dry ice to the US, where specimens were stored at −80°C for less than 6 months. Women in this sample did not report current or past hormonal contraceptive use.

Tsimane urinary testosterone was analyzed at the University of California-Santa Barbara (UCSB) Human Biodemography Laboratory using an in-house enzyme immunoassay based on R156/7 (C. Munro, UC-Davis) (Muir et al., 2001). The within and between coefficients of variation (CV) were 3.7% and 7.8% for the high (1339 pg/mL) and 3.8% and 6.8% for the low (307 pg/mL) controls. Urine specimens were corrected for specific gravity with a hand-held refractometer (Atago, Inc.) (Miller et al., 2004). Mean specific gravity-corrected urinary testosterone across the 4–6 days of specimen collection was used for analysis.

2.2 | Hadza data collection

Following the same protocol as above, $n = 11$ Hadza males aged 18–65 (mean age 34.5) drank DLW and collected urine specimens to measure TEE (Pontzer et al., 2015). Hadza urinary testosterone was analyzed at the University of New Mexico with an in-house enzyme immunoassay also based on R156/7 (C. Munro, UC-Davis) (Muir et al., 2001). Testosterone in urine samples was first deconjugated by treatment with beta-glucuronidase (*Helix pomatia*, Calbiochem, <2% aryl-sulfatase) and ether extraction. The interassay CVs were 5.9% for the low and 11.0% for the high controls; within plate CVs were 6.2%. Specific gravity corrected urinary testosterone was treated as above to create summary measures.

2.3 | TEE analysis

TEE (kcal/day) was calculated from urinary ^{18}O and ^2H abundance using cavity ring down spectrometry (L2120i, Picarro Inc., Santa Clara, CA) at the Hunter College Human Evolution and Energetics Laboratory as described previously (Pontzer, Durazo-Arvizu, et al., 2016). Dilution spaces were calculated using the slope intercept method, while the rate of CO_2 production was calculated using equation 17.15 in (Speakman, 1997). Food quotients (Tsimane: 0.93, Hadza: 0.86) were used to convert CO_2 production into TEE using the modified Weir equation (Gurven, Trumble, et al., 2016). Note that any differences or errors in population food quotients will affect

differences in population means for TEE but will not affect variance in TEE within each population, which is the focus of these analyses. Body water, calculated as for dilution space, was used to calculate fat free mass, and thus body fat percentage, assuming a hydration constant of 0.732.

2.4 | Accelerometry data

Tsimane DLW study participants also wore an accelerometer (Actigraph wGT3X, Actigraph LLC, Pensacola, FL) to quantify physical activity level during the period of TEE measurement. Participants removed the accelerometer to bathe, but otherwise wore the device at all times while engaging in routine activities. Participants wore the accelerometer for 2.5 days, on average. Daily step counts were used as a proxy to estimate physical activity (see Gurven et al., 2013 for additional details). Height was measured with a portable stadiometer (Seca 213), weight was measured on a Tanita BC1500 Scale, and fat free mass was determined using isotopic dilution; range and median values for anthropometrics are shown in Table 1. Hadza participants did not wear accelerometers.

2.5 | Data analysis

Data were analyzed in STATA 17 (College Station, TX) and R 3.3.2. There are empirical and theoretical reasons for stratifying the association between testosterone and TEE by sex. Empirically, Tsimane men had 48.5% higher testosterone ($t = 5.20$, $p < .001$), 28.8% greater fat free mass ($t = 5.20$, $p < .001$), and 14% higher accelerometry counts ($t = 1.96$, $p = .029$) than women. From a theoretical perspective, one would not expect the same associations between testosterone and TEE given the differential costs of reproduction and variance in reproductive strategy across the sexes (Gurven, Costa, et al., 2016; Stieglitz et al., 2019); female life-history strategies do not rely on testosterone-mediated behavior or muscle mass, while male strategies can benefit from high testosterone. Tsimane men engage in more intense physical activity due to sexual division of labor, including hunting and chopping down trees (Gurven et al., 2013; Gurven, Trumble, et al., 2016). Additionally, males and females have different distributions of both testosterone, and TEE, resulting in significant heteroscedasticity, necessitating separate analyses. Hadza female testosterone was not measured.

Variance inflation factors (VIF) were utilized to assess covariance and insure minimal collinearity in regression models (all VIF < 2.5). Testosterone was logged to induce normality, and maximum daily step counts were used as a proxy for physical activity for the Tsimane data. In four cases, accelerometry measures were not available, and for each model Bayesian imputation methods were used in the regression analysis via Hamiltonian MCMC map2-stan package (version 1.59).

2.6 | Ethics

Informed consent was collected at three levels: by the individual, by the community, and by the Tsimane Gran Consejo (Tsimane governing body). All study protocols were approved by the Institutional Review Boards of the University of New Mexico (# 07-157) and the University of California Santa Barbara (#3-21-0652).

2.7 | Comparisons across populations

TEE data for both Tsimane and Hadza were analyzed at the Hunter College Human Evolution and Energetics Laboratory following the exact same protocols, and thus TEE is directly comparable across populations (Kraft et al., 2021). While the same antibodies were used to measure testosterone in both laboratories, and thus there is minimal risk of cross-reactivity, testosterone was analyzed directly in one lab and after extraction of metabolites in the other (Travison et al., 2017). To facilitate testosterone-TEE comparisons across populations are

TABLE 1 Sample characteristics—median and range for study variables by sex and population.

	Tsimane female ($N = 20$)	Tsimane male ($N = 20$)	Hadza male ($n = 11$)
Age	48.5 (18–87)	47.5 (20–77)	30.0 (18–65)
TEE (kcal/day)	2186 (1568–2861)	3065 (2340–4057)	2685 (2008–3363)
Fat free mass (kg)	35.6 (25–44)	49.0 (41–62)	44.8 (39.7–49.7)
Body fat (%)	31.8 (17–40)	23.2 (12–30)	13 (7–23)
Max step counts/day	22 715 (9624–41 849)	27 224 (15 274–45 369)	NA
BMI (kg/m^2)	22.7 (17.8–31.1)	24.9 (20.6–26.9)	19.9 (19.0–23.4)

TABLE 2 Association between TEE (kcal/day) and testosterone for $n = 40$ Tsimane men and women.

	Tsimane male ($n = 20$)						Tsimane female ($n = 20$)					
	Model 1		Model 2		Model 3		Model 4		Model 5		Model 6	
Predictor	Std. beta	*p* value	Std. beta	*p* value	Std. beta	*p* value	Std. beta	*p* value	Std. beta	*p* value	Std. beta	*p* value
Log testosterone	.42	.025	.41	.018	.44	.004	.08	.373	−.02	.546	−.06	.66
Fat free mass (kg)			.57	.001	.68	<.001			.71	<.001	.75	<.001
Accelerometry max					.26	.099					.3	.04
WAIC	57.2		49.3		49.2		61.5		49.5		46.5	
Model R^2	0.17		0.47		0.53		0.04		0.45		0.54	

conducted by analyzing population specific *Z*-scores to understand the relative association between testosterone and TEE, see limitations. Not all variables were available for both populations, so a reduced model was used for comparative analyses.

3 | RESULTS

3.1 | Tsimane

3.1.1 | Testosterone, fat free mass, and physical activity

Controlling for age, log testosterone was positively associated with lean mass (Std. Beta = .48, $p = .02$). Log testosterone was positively albeit not significantly associated with physical activity (as measured by accelerometry).

3.1.2 | Male association between testosterone and energetic expenditure

For Tsimane men, testosterone was positively associated with TEE (Std. $\beta = .44$, $p = .004$) controlling for fat free mass and physical activity (Table 2: Model 3, Figures 1 and 2). A one standard deviation (SD) in testosterone was associated with an additional expenditure of 240 calories/day, or approximately 7.8% (range 5.9%–10.2%) of TEE.

After controlling for fat free mass, age was not associated with men's TEE ($p = .660$), nor was it associated with men's testosterone ($p = .206$), so age was not included in the models. Fat free mass was not associated with physical activity in men ($p = .923$). Testosterone had a larger association than physical activity on male TEE (Std. $\beta = .44$ for testosterone, Std. $\beta = .26$ for physical activity) controlling for body composition.

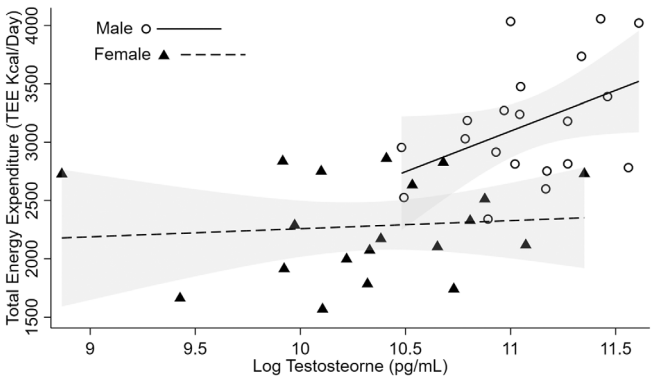

FIGURE 1 Association between TEE (kcal/day) and testosterone for $n = 40$ Tsimane men and women.

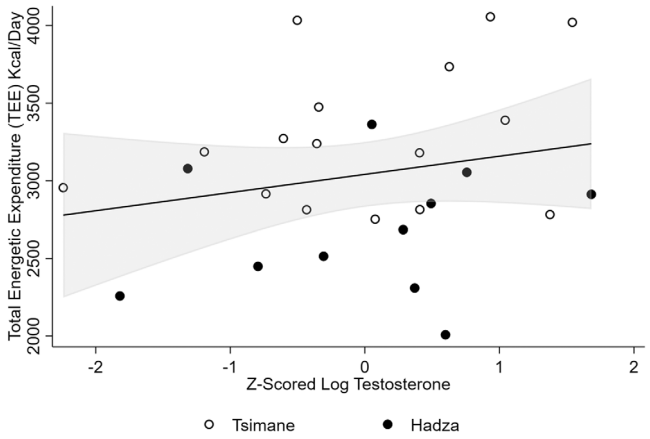

FIGURE 2 Association between male TEE (kcal/day) and testosterone for $n = 20$ Tsimane and $N = 11$ Hadza.

3.1.3 | Female association between testosterone and energetic expenditure

There was no association between testosterone and TEE in women (Table 2: Models 4–6, Figure 1); a one SD difference in female testosterone was associated with an

additional 39 calories (NS). Fat free mass was not associated with physical activity in women ($p = .448$), and the association between testosterone and fat free mass was not significant (Std. $\beta = .09$, $p = .700$, $r^2 = .01$). Adding fat free mass to the model did not change this association (Std. $\beta = -.02$, $p = .888$, $r^2 = .53$). Age was not associated with TEE, and not included in these models.

3.1.4 | Hadza and Tsimane comparison

Due to small sample size ($n = 11$), the Hadza sample did not have adequate statistical power to assess the relationship between testosterone and TEE; a post hoc power analysis suggests a sample size of $n = 38$ Hadza men would be required to have sufficient power. Though Hadza analyses did not adjust for physical activity, the T-TEE relationship in Tsimane was robust in models that did not adjust for physical activity (Table 2). A regression model finds a positive (though non-significant) association between Hadza testosterone and TEE controlling for fat free mass, with a one standard deviation difference in testosterone equivalent to an additional 96 calories burned per day.

A combined model including all Hadza and Tsimane men ($n = 31$) indicates that a one standard deviation difference in testosterone is associated with an additional 177 calories per day ($p = .017$), controlling for fat free mass with a dummy variable for population (see Table 3, Figure 2). There was not a significant interaction between population and testosterone.

4 | DISCUSSION

Here we provide evidence in humans from two free-living subsistence populations that endogenous testosterone is associated with higher total energy expenditure. Across vertebrates, testosterone plays a critical role in determining how males expend and allocate energy, particularly in the context of reproduction (Bribiescas, 2010; Folstad & Karter, 1992; Muehlenbein & Bribiescas, 2005). High testosterone levels promote investments in body growth and muscle mass, ornaments of status, and costly forms of behavioral mating effort, all of which come with energetic costs. A major focus of literature on sexual selection has therefore investigated how these investments trade-off against other functions, particularly the survival-enhancing functions of immunity and cellular repair (Bribiescas, 2010; Folstad & Karter, 1992; Muehlenbein & Bribiescas, 2005). This has raised the possibility that testosterone levels are elevated primarily in those individuals that can best afford to pay the costs. This could explain why men in subsistence level populations produce substantially lower levels of testosterone than men in industrialized populations (Bribiescas, 1996; Ellison et al., 2002; Trumble et al., 2012; Vitzthum et al., 2009). While many studies have focused on associations between testosterone and lean mass, or the acute impacts of physical activity, few have measured the energetic costs above and beyond lean mass or physical activity associated with testosterone in free-living humans participating in normal daily activities.

We find that a one standard deviation difference in testosterone is associated with 240 calories of energetic expenditure per day among Tsimane men and 96 calories for Hadza men (an average of 177 calories across both populations). For comparison, the difference in TEE for adults with sedentary versus active lifestyles in a larger sample ($n = 332$) across several populations was only ~200 calories per day (Pontzer, Durazo-Arvizu, et al., 2016), indicating that a one standard deviation in testosterone is as costly as the difference between sedentary and active lifestyles. Indeed, the association between testosterone and TEE among Tsimane men is greater than the association of physical activity (Table 2, Model 3), although we note that the magnitude of the testosterone effect and relative size of the effect compared to physical activity could differ in other populations, and it should also be noted that physical activity is also difficult to measure accurately (see limitations). It should be noted that across populations, the relationship between energetic expenditure and physical activity is relatively weak in general (Kraft et al., 2021; Pontzer, 2015; Pontzer, Durazo-Arvizu, et al., 2016).

TABLE 3 Association between TEE (kcal/day) and testosterone for $n = 20$ Tsimane men and $N = 11$ Hadza men with a limited set of covariates that were collected in both populations.

	Tsimane $n = 20$		Hadza $n = 11$		Combined $n = 31$	
	Coef	*p*-value	Coef	*p*-value	Coef	*p*-value
Testosterone (*Z* score)	219.4	.018	96.2	.490	177.3	.017
Fat free mass (kg)	43.0	.003	41.5	.262	43.2	.001
Population (binary, Tsimane)					273.2	.080
R^2	0.53		0.18		0.54	

The anabolic effects of testosterone increase protein synthesis (Brodsky et al., 1996; Griggs et al., 1989), as well as muscle cell proliferation via satellite cell activation (Sinha-Hikim et al., 2006), both of which require intensive caloric utilization and diversion of energy stores. Testosterone also increases glucose uptake and utilization in muscle tissue (Tsai & Sapolsky, 1996). The caloric costs of a high testosterone phenotype may help explain why men taking prescription testosterone supplements often show decreases in fat mass in addition to increases in muscle mass (Emmelot-Vonk et al., 2008; Tenover, 1992; Wittert et al., 2003), while androgen deprivation therapy results in increased fat mass (Smith, 2004). Indeed, lower testosterone in late adulthood is often associated with higher body fat (Derby et al., 2006; Shi et al., 2013; Svartberg et al., 2003; Wu et al., 2008) and reduced muscle mass (Iannuzzi-Sucich et al., 2002).

Women also have testosterone and lean muscle, but do not show the same associations between TEE and testosterone. There are several possible reasons why female testosterone is not associated with energetic expenditure. First, women pay additional costs of reproduction (gestation, lactation) to which men are not exposed (Gurven, Costa, et al., 2016) and which may overwhelm or obfuscate the relationship between testosterone and TEE. Women in this study were not pregnant, they did vary in reproductive status (age range 18–87), and cycling women face additional costs that menopausal women do not. Second, women have significantly lower levels of testosterone and lower levels of muscle mass, do not engage in the same levels of physical activity (Gurven et al., 2013), and have lower energy expenditures than Tsimane men (Gurven, Trumble, et al., 2016).

While most research on human male testosterone has been conducted in industrialized populations, subsistence populations facing higher infectious burden and less food security show lower levels of testosterone compared to age-matched males in industrialized populations, even after accounting for differences in body size (Bribiescas, 1996; Ellison et al., 2002; Trumble et al., 2012; Vitzthum et al., 2009). These lower levels of testosterone may contribute to population differences in a number of health outcomes, including lower levels of prostate enlargement (Trumble et al., 2015), and lower prostate cancer risk (Alvarado, 2010). The association between relatively low levels of testosterone and these lower health risks raises the possibility that men living in industrialized populations pay significantly higher energetic costs to maintaining their relatively high testosterone phenotypes.

Studies in humans and other vertebrates have indicated that TEE is broadly similar across populations despite substantial differences in physical activity and lifestyle (Pontzer, 2015; Pontzer et al., 2012, 2015; Pontzer, Brown, et al., 2016). Tsimane and Hadza are highly physically active and have exceptional cardiovascular fitness (Gurven et al., 2012; Kaplan et al., 2017; Raichlen et al., 2017; Rowan et al., 2021). Tsimane men engage in intensive tree chopping to clear horticultural plots (Trumble et al., 2013), as well as active hunting in dense tropical forests (Trumble et al., 2014), while Hadza men often walk ~20 km or more on hunts across the savanna, and climb and chop into trees to harvest honey (Raichlen et al., 2014; Wood & Marlowe, 2013). Yet Hadza TEE is comparable to sedentary, socioeconomically developed populations, and Tsimane TEE is only marginally higher than industrialized populations. Moreover, the elevation in Tsimane TEE is attributable to their elevated RMR, which in turn reflects their high levels of parasitic exposure and immune activation (Blackwell et al., 2016; Gurven, Trumble, et al., 2016). In analyses controlling for body size and RMR, Tsimane TEE is not elevated relative to more sedentary, developed populations, and in fact the ratio of TEE/RMR, often used as an index of energy expended in physical activity, is comparatively low (men: 1.56, women: 1.41) (Gurven, Trumble, et al., 2016). Low testosterone levels among the Tsimane and Hadza may act to mitigate the effects of physical activity on TEE, effectively reducing their TEE to levels seen in less active populations. More broadly, the lower levels of testosterone reported in physically active subsistence populations (Bribiescas, 1996; Ellison et al., 2002; Trumble et al., 2012; Vitzthum et al., 2009) may contribute to the similarities in TEE across populations (Blackwell et al., 2016; Pontzer, 2015; Pontzer et al., 2012; Pontzer, Durazo-Arvizu, et al., 2016) and the poor correspondence between levels of physical activity and TEE.

4.1 | Limitations

A small sample size limits the power and generalizability of this study. Unfortunately given the costs and logistics of DLW studies, most studies of TEE in free-living populations are relatively small, especially those conducted across several remote non-industrial populations (Kraft et al., 2021; Pontzer et al., 2015). Thus, while some of the findings do not reach statistical significance depending on the covariates included in the model, the results show a clear association regardless of the variables utilized across two populations. Not all of the same covariates were collected in both populations, so our comparisons are limited in scope. Total energetic expenditure was measured in the same lab and thus directly comparable, but testosterone was measured in two different labs, though using the same antibodies with the same cross-

reactivities. In order to assess whether the same general association exists between populations, we *Z*-scored testosterone individually by population, and then we pooled the populations to run a regression model (Table 3); a visualization of these model results are shown in Figure 2. While hormonal data are generally not comparable across labs (Rosner et al., 2007), these models are only assessing whether the same associations between testosterone and TEE are similar across populations (not comparing testosterone directly across populations), which is especially important given the dearth of cross-population data from subsistence populations.

Hormonal regulation of RMR and TEE remains poorly documented in humans (Bribiescas, 2010; Leonard, 2012; Muehlenbein & Bribiescas, 2005), and other critical metabolic hormones such as cortisol, progesterone, adiponectin, and ghrelin need to be examined in future studies to better understand what factors are associated expenditures in both men and women. Due to the logistics, time, and resource-intensive nature of accelerometer data collection, physical activity data were only measured for a subset of the days (mean 2.5 days) over which the DLW data was collected. While DLW is minimally invasive, wearing an accelerometer 24-h a day for multiple days is difficult, and collecting a full week of accelerometry data would be unacceptable to most participants; it should be noted that physical activity is difficult to measure with no gold standard. While accelerometry is an objective measure that captures daily activity without relying on participant recall, it may not accurately assess all types of physical activity. Additionally, information on cycle day was not collected for Tsimane women (though it should be noted that half of the women in this sample were post-reproductive).

An observational study such as this one cannot establish direct causality between testosterone and increased energetic expenditure. While we control for some measures of phenotypic condition (such as anthropometric status and physical activity), there is likely still some level of phenotypic correlation; men in better condition can afford higher levels of testosterone and also maintain higher levels of energetic expenditure. That said, the analyses presented here show, an albeit weakened, effect of testosterone on TEE controlling for both muscle mass and physical activity. This suggests that a high testosterone phenotype is energetically expensive above and beyond the costs of maintaining muscle mass; the costs of a high testosterone phenotype likely include other unobserved physiological processes.

Physical activity induces acute increases in testosterone, which are then cleared by the kidneys and later excreted in urine. However, these acute increases usually subside within 1–2 h after physical exertion (Jensen et al., 1991), and so it seems unlikely that these acute increases in testosterone would impact first morning void urine concentrations. The association between testosterone and energetic expenditure was attenuated among male Hadza hunter-gatherers (Pontzer et al., 2015). It is possible that this was due to a smaller sample size of Hadza males and thus low statistical power, different age range of participants, parasite and pathogen exposures (Gurven, Trumble, et al., 2016), or that the types and intensity of physical activity due to different subsistence strategies result in different body mass and testosterone profiles across these populations. Indeed Hadza have lower TEE than Tsimane (Pontzer et al., 2015), and lower levels of C-reactive protein, a non-specific marker of immune activation (Raichlen et al., 2017). Thus differences in immune costs may play a role in the variation in both TEE and the immune-related costs of testosterone across populations living in different immune environments (Gurven, Trumble, et al., 2016; Trumble et al., 2016).

5 | CONCLUSIONS

Men with higher testosterone had higher total energy expenditure. This suggests that a high testosterone phenotype is associated with energetic expenses; the costs of a high testosterone phenotype likely include both energetically expensive muscle mass, and other unobserved physiological processes such as increased glucose uptake (Tsai & Sapolsky, 1996). Women's energy expenditure was not associated with testosterone, perhaps due to differential costs of reproduction in men and women, which warrants future investigation. A high testosterone phenotype has caloric costs such as mediating the distribution of energetic resources toward reproduction or maintenance. These results demonstrate an energy cost of a high testosterone phenotype in human males, while beneficial for reproduction, is also energetically expensive.

AUTHOR CONTRIBUTIONS

Benjamin C. Trumble: Conceptualization (lead); data curation (lead); formal analysis (lead); funding acquisition (equal); investigation (equal); methodology (equal); project administration (equal); resources (equal); visualization (lead); writing (lead); review & editing (lead). **Herman Pontzer**: Conceptualization (equal); data curation (equal); investigation (equal); methodology (equal); resources (equal); review & editing (equal). **Jonathan Stieglitz**: Funding acquisition (equal); investigation (equal); methodology (equal); project administration (equal); resources (equal); review & editing (equal). **Daniel K. Cummings**: Data Curation (equal); investigation

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ACKNOWLEDGMENTS

We thank the Tsimane and Hadza participants, Jason Marks, Carmen Mavis Ardaya, Ivan Maldonado Suarez and the Tsimane communities for their hospitality. Lauren Christopher, Khalifa Stafford, and Jenny Paltan assisted in isotope analyses.

FUNDING INFORMATION

National Institute on Aging within the National Institute of Health (RF1AG054442). National Science Foundation (1748282). Jonathan Stieglitz acknowledges the French National Research Agency under the Investments for the Future (Investissements d'Avenir) programme (ANR-17-EURE-0010).

CONFLICT OF INTEREST STATEMENT

No authors report any conflicts of interest.

DATA AVAILABILITY STATEMENT

Individual-level data are stored in the Tsimane Health and Life History Project (THLHP) Data Repository, and are available through restricted access for ethical reasons. THLHP's highest priority is the safeguarding of human subjects and minimization of risk to study participants. The THLHP adheres to the "CARE Principles for Indigenous Data Governance" (Collective Benefit, Authority to Control, Responsibility, and Ethics), which assure that the Tsimane (1) have sovereignty over how data are shared, (2) are the primary gatekeepers determining ethical use, (3) are actively engaged in the data generation, and (4) derive benefit from data generated and shared for use whenever possible. The THLHP is also committed to the "FAIR Guiding Principles for scientific data management and stewardship" (Findable, Accessible, Interoperable, and Reusable). Requests for individual-level data should take the form of an application that details the exact uses of the data and the research questions to be addressed, procedures that will be employed for data security and individual privacy, potential benefits to the study communities, and procedures for assessing and minimizing stigmatizing interpretations of the research results (see the following webpage for links to the data sharing policy and data request forms: https://tsimane.anth.ucsb.edu/data.html). Requests for individual-level data will require institutional IRB approval (even if exempt) and will be reviewed by an Advisory Council composed of Tsimane community leaders, community members, Bolivian scientists, and the THLHP leadership. The study authors and the THLHP leadership are committed to open science and are available to assist interested investigators in preparing data access requests.

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How to cite this article: Trumble, B. C., Pontzer, H., Stieglitz, J., Cummings, D. K., Wood, B., Emery Thompson, M., Raichlen, D., Beheim, B., Yetish, G., Kaplan, H., & Gurven, M. (2023). Energetic costs of testosterone in two subsistence populations. *American Journal of Human Biology*, e23949. https://doi.org/10.1002/ajhb.23949