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ORIGINAL ARTICLE

Testosterone, 8-Oxo-2′-Deoxyguanosine (8-OHdG) and Cu/Zn Superoxide Dismutase (SOD) in Adult Shuar Males of Amazonian Ecuador: A Test for Evidence of Trade-Offs Between Reproductive Effort and Oxidative Stress

R. G. Bribiescas[1] | A. Sancilio[2] | D. Amir[3] | T. J. Cepon-Robins[4] | T. Gildner[5] | M. A. Liebert[6] | F. Madimenos[7] | S. S. Urlacher[8] | J. Snodgrass[9] | L. Sugiyama[9]

[1]Department of Anthropology, Yale University, New Haven, Connecticut, USA | [2]3EO Health, Beverly, Massachusetts, USA | [3]Department of Psychology, Duke University, Durham, North Carolina, USA | [4]Department of Anthropology, University of Colorado, Colorado Springs, Colorado, USA | [5]Department of Anthropology, Washington University, St. Louis, Missouri, USA | [6]Department of Anthropology, Northern Arizona University, Flagstaff, Arizona, USA | [7]Department of Anthropology, Queens College, City University of New York, New York, New York, USA | [8]Department of Anthropology, Baylor University, Waco, Texas, USA | [9]Department of Anthropology, University of Oregon, Eugene, Oregon, USA

Correspondence: R. G. Bribiescas (richard.bribiescas@yale.edu)

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ABSTRACT

Objectives: Reproductive effort incurs the cost of biological aging and morbidity by compromising somatic maintenance when key resources are limited. Oxidative stress is positively correlated with reproductive effort in adult human females and non-human male animal models, but human males are understudied. We hypothesized that due to its anabolic and metabolic promotion of reproductive effort in human males, testosterone would be positively associated with biomarkers of oxidative stress.

Methods: Urinary testosterone in adult Shuar males of Amazonia Ecuador, a foraging/horticultural population, was measured with urinary 8-hydroxy-2′ -deoxyguanosine (8-OHdG), a biomarker of oxidative stress, and Cu/Zn superoxide dismutase (Cu/Zn SOD), a protective antioxidant against oxidative stress. Age and anthropometric measures were included in multivariate models.

Results: No significant correlation was observed between testosterone and 8-OHdG, $r^2 = 0.01$, $p = 0.61$, $n = 29$, or Cu/Zn SOD, $r^2 = 0.0005$, $p = 0.93$, $n = 17$. Multiple linear regression models including testosterone, Cu/Zn SOD, anthropometrics, and age, with 8-OHdG as the dependent variable, were modestly supportive of an association. The most parsimonious 8-OHdG model included age, Cu/Zn SOD, and testosterone ($R^{2\ \text{adjusted}} = 0.38$, $p = 0.04$, AICc = 141.95). All multivariate models for Cu/Zn SOD were not significant ($p > 0.05$).

Conclusions: Oxidative stress may not be a cost of reproductive effort in this population of adult males; perhaps due to consistently low testosterone levels in non-industrialized populations, differences in the metabolic cost of reproductive effort between males and females (i.e., aerobic metabolism), and/or study limitations based on cross-sectional measures of oxidative stress and testosterone.

1 | Introduction

Reproductive effort is often accompanied by negative effects on somatic maintenance and survivorship, especially when key resources are limited, such as caloric energy (Ellison 2003; Hirshfield and Tinkle 1975; Jasienska 2009; Stearns 1989). This life history trade-off is evident for both sexes in non-human animal models (Oakwood et al. 2001; Partridge and Farquhar 1981) but is challenging to observe in humans due to methodological constraints, social factors, and resource abundance in wealthy societies (Ellison 2003; Jasienska 2009). Demographic evidence of trade-offs between reproductive effort and mortality has been demonstrated in post-menopausal human females through negative associations between gravidity and lifespan. The number of sons and daughters was negatively associated with maternal lifespan, entailing an 18 months decrease in mother's lifespan per offspring (Jasienska et al. 2006). Similar trade-offs in other populations and time periods have been demonstrated across numerous studies (Cesarini et al. 2009; Cesarini et al. 2007; Helle et al. 2002; Helle et al. 2010). However, the energetic status of populations as measured hormonally or through anthropometrics is often not taken into consideration. This contributes to mixed results from studies that test for the presence of a trade-off between reproductive effort and morbidity, mortality, and/or aging (Jasienska 2009); also see Jasienska (2020) for a review.

Similar trade-offs between reproduction and mortality are not well documented for human males. While parity does not contribute to direct somatic costs in males (Jasienska et al. 2006), reproductive effort in human males is expressed differently compared to females. In males, including humans, reproductive effort is often evident through behavioral dimorphism and the lifetime metabolic costs of sexually dimorphic tissue (Ellison and Bribiescas 2018). Testosterone accentuates and manages behavioral and somatic reproductive effort in males, including humans (Bribiescas 2001; Bribiescas et al. 2012; Ellison 2003). Testosterone promotes reproductive behavior (i.e., libido) and contributes to differences in body composition including greater sexually dimorphic muscle anabolism and metabolism compared to females (Bhasin et al. 2001a, 2001b; Sattler et al. 2009). Testosterone also contributes to adipose tissue catabolism and increased metabolic costs both in vitro and in vivo under varied ecological and lifestyle conditions (Trumble et al. 2023; Tsai and Sapolsky 1996; Welle et al. 1992).

Lifetime metabolic costs have been negatively associated with lifespan and increases in mortality in a large cohort of human adult males (Austad 2006; Ruggiero et al. 2008). Testosterone-induced increases in metabolic investment may contribute to morbidity and mortality, specifically through increases in oxidative stress. Oxidative stress is the result of toxic byproducts produced during aerobic metabolism. The production of reactive oxidative species (ROS), free radicals, and peroxides requires clearance and/or deactivation. Exposure to these agents results in genetic, cellular, and tissue damage and accelerated aging (Adelman et al. 1988; Dowling and Simmons 2009; Finkel and Holbrook 2000). The imbalance between untreated ROS, free radicals, and peroxides compared to protective and repair mechanisms is defined as oxidative stress. Oxidative stress contributes to morbidity and variation in biological aging and mortality across species that utilize aerobic metabolism (Adelman et al. 1988; Dowling and Simmons 2009; Finkel and Holbrook 2000; Frisard and Ravussin 2006).

Higher basal metabolic rates can result in greater oxidative stress. This is evident between and within species. Organisms with greater body mass tend to have lower basal metabolic rates (BMR) and less exposure to oxidative stress (Adelman et al. 1988). While there are important exceptions to this pattern (Austad 1997; Brunet-Rossinni and Austad 2004; Saldmann et al. 2019), the overall association is robust. In humans, individual variation in oxidative stress is often positively associated with metabolic rates, although most data are from children and adolescents (Matusik et al. 2015; Redman et al. 2018; Samsonov and Urlacher 2025; Topp et al. 2008). Conditions that accelerate metabolic rates, such as hyperthyroidism, have also been associated with increases in oxidative stress (Aslan et al. 2011). Specific tissues that rely heavily on aerobic metabolism, such as the brain, also exhibit associations between metabolism and oxidative stress (Lushchak et al. 2021).

Oxidative stress is also an important proximate cost of reproductive effort that can contribute to life history trade-offs (Dowling and Simmons 2009; Monaghan et al. 2009). As organisms invest in aerobic metabolism that supports tissues and behavior that reflect reproductive effort, the production of ROS, free radicals, and peroxides can manifest in the form of oxidative stress when protective mechanisms are at capacity (Carlos Alonso-Alvarez et al. 2004; Speakman and Garratt 2014). Also see Metcalfe and Monaghan (2013) (Figure 1).

This reproductive model is complicated by tissue-specific variation in oxidative stress in various organisms, the possibility of phenotypic correlations that result in investment in both reproductive effort and OS protective mechanisms, as well as limitations on the experimental manipulation of reproductive effort to effectively test for a trade-off (Costantini 2019; Jasienska 2009; Metcalfe and Monaghan 2013). Figure 2 illustrates additional sources of variation when considering oxidative stress/reproductive effort trade-offs, including the potential contribution of reproductive hormones and OS protective mechanisms. Testosterone can increase reproductive effort through greater metabolism and therefore also increase oxidative stress. This has been demonstrated in non-human animal models, including non-human primates (Alonso-Alvarez et al. 2007; Austad 2006; Bergeron et al. 2011; Bueno et al. 2017; Costantini 2008; Georgiev et al. 2015; Gonzalez et al. 2020; Videan et al. 2009). The incorporation of reproductive hormones and tissue-specific oxidative stress has been presented as a more accurate model of the potential cost of oxidative stress in response to reproductive effort (Figure 2).

1.1 | 8-OHdG

8-Hydroxy-2′-deoxyguanosine (8-OHdG) is an informative and widely used biomarker of oxidative stress (Valavanidis et al. 2009). It reflects the number of repaired genetic lesions due to oxidative damage at the guanine base pair of DNA. When such lesions occur, the DNA repair enzyme oxoguanine glycosylase (OGG1) excises the damaged base pair, oxidizes it, and then combines with a hydroxyl group before being cleared in urine as

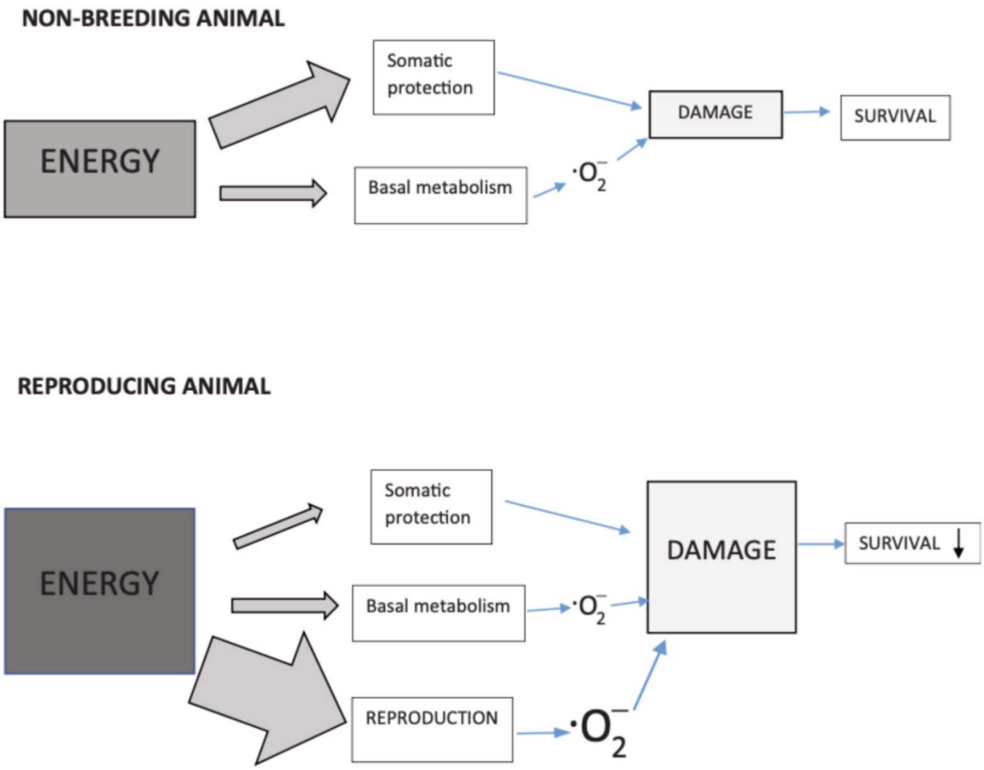

FIGURE 1 | A basic model contrasting the role of oxidative stress in a non-breeding and reproducing individual. Size of gray arrows reflects variation in energy allocation. O_2^- = reactive oxidative species (Speakman and Garratt 2014).

8-OHdG. The advantages of 8-OHdG as a measure of oxidative stress include ease of measurement in urine using commercial enzyme-linked assays, non-invasive collection of urine samples, robust storage and handling tolerances of samples, and the specificity of reflecting repaired oxidative stress damage to DNA. It is therefore a useful biomarker of the physiological cost of oxidative stress in that it reflects damage as well as investment in repair. 8-OHdG is also unaffected by diet or dietary sources of antioxidants, which can affect the expression and measurement of other oxidative stress measures (Gackowski et al. 2001).

1.2 | Cu/Zn SOD

Superoxide dismutase (SOD) is an antioxidant metalloprotein that catalyzes superoxides and free radicals into ordinary oxygen (O_2) and hydrogen peroxide (H_2O_2), thereby protecting against tissue, cellular, and genetic damage resulting from oxidative stress (McCord and Fridovich 1969). Several metalloprotein variants of SOD, with copper/zinc [Cu/Zn] SOD being the common form, are found in eukaryotic organisms, including humans, although the manganese variant (Mn SOD) is located within human mitochondria. Three genetic variants of SOD are produced in different areas of the body: Cu/Zn SOD1 in the cytoplasm, Mn SOD2 in mitochondria, and Cu/Zn SOD3 in the extracellular fluid. The Cu/Zn SOD3 form (hereon identified simply as Cu/Zn SOD) is the measured variant in urine by commercial assays such as in the present investigation. Cu/Zn SOD deficiency in mice and humans results in extreme oxidative stress damage leading to shortened lifespans due to neuromuscular dysfunction, including a higher risk of amyotrophic lateral sclerosis (ALS), Parkinson's disease, cardiovascular disease, and cancer (Johnson and Giulivi 2005; Miao and St Clair 2009). Measurements of Cu/Zn SOD are therefore useful assessments of the deployment of protective mechanisms in response to the production of superoxides and free radicals. Although Cu/Zn SOD is more commonly measured in serum and plasma under clinical conditions, urinary measurements using enzyme assays have been demonstrated to reflect health status in humans, including elevated levels in renal function and in individuals with Down's Syndrome (Nishimura et al. 1982; Porstmann et al. 1988). While urinary measurements of any biomarker seldom correlate well with circulating levels in blood within individuals due to the time lag of cumulative renal and hepatic clearance, SOD levels are comparable in urine and blood (Adachi et al. 1992; Nishimura et al. 1982).

1.3 | Reproductive Effort and Oxidative Stress

Associations between urinary 8-OHdG and Cu/Zn SOD with gravidity and parity have been reported in rural Polish women (A. Ziomkiewicz et al. 2016). However, associations between OS and reproductive effort can vary depending on ecological context. While oxidative stress was positively associated with gravidity in rural Polish women, no association was evident in urban American women (Ziomkiewicz et al. 2018). Sancilio et al. (2021) also reported 8-OHdG and Cu/Zn SOD to be modestly associated with reproductive effort in urban and rural Polish women, with rural women exhibiting higher levels of 8-OHdG and Cu/Zn SOD compared to urban women. Research on the association between hormonal measurements of reproductive effort and oxidative stress in males has largely been limited to non-human animal models (C. Alonso-Alvarez et al. 2007).

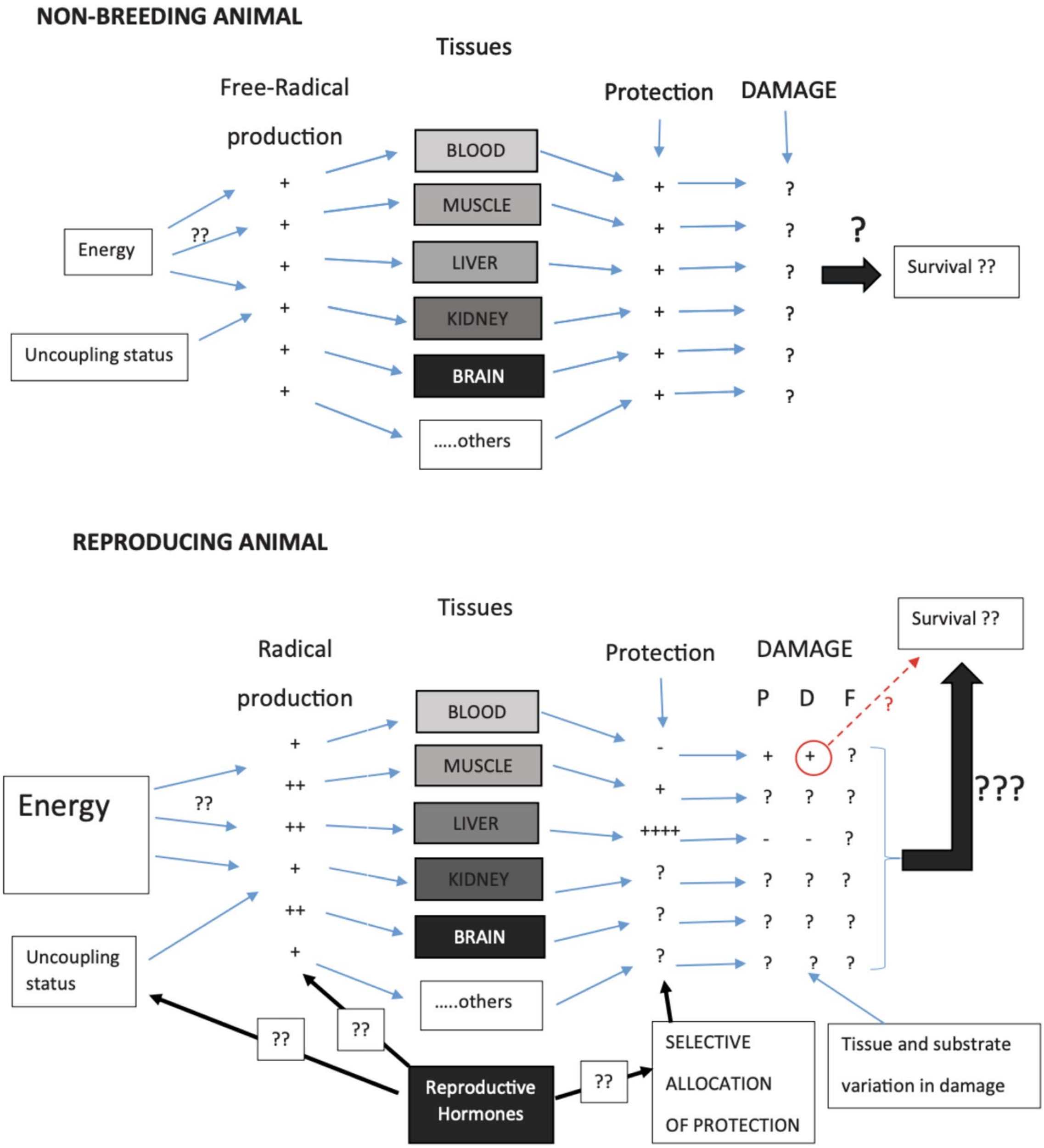

FIGURE 2 | A more detailed model of the association between tissue specific oxidative stress, reproductive hormones, protective mechanisms, and survivorship in a non-breeding and reproducing individual. P = protein, D = DNA, and F = fats (Speakman and Garratt 2014). Note the unclear association between reproductive hormones and protective mechanisms/agents contributing to variation in tissue damage from oxidative stress.

Only a few studies have examined 8-OHdG, Cu/Zn SOD, and testosterone in adult human males (Christensen et al. 2024; Ishikawa et al. 2007; Yasuda et al. 2008), and none have been from non-industrialized societies. Skogastierna et al. (2014) reported significant increases in oxidative stress and nitric oxide levels in healthy adult human males administered supraphysiological doses of testosterone in vivo, as well as in vitro with vascular endothelial cells exposure to testosterone. Based on this evidence, we developed and tested the following hypotheses.

In Figure 3, we present a model of what we determine to be the predicted associations between the variables in this investigation.

Based on these predictions, we tested the following hypotheses.

H1. *Due to its positive effect on metabolism and anabolism, testosterone will be positively associated with 8-OHdG as a measure of oxidative stress.*

H2. *Testosterone will be positively associated with Cu/Zn SOD due to the deployment of protective responses to greater oxidative stress resulting from testosterone.*

H3. *If Cu/Zn SOD responses are robust, there will be an inverse relationship between Cu/Zn SOD and 8-OHdG due to decreases in* ROS.

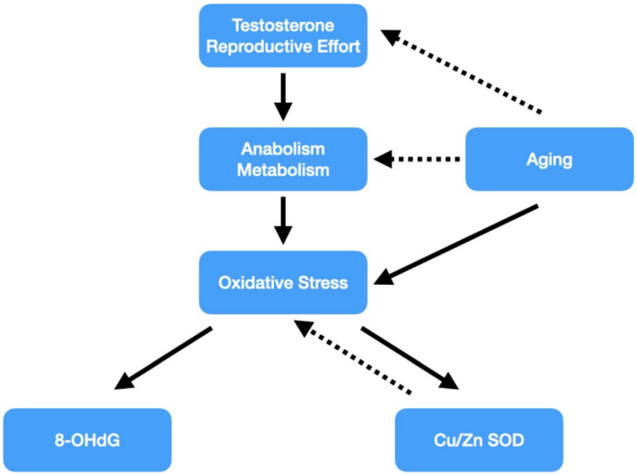

FIGURE 3 | Based on our current understanding of oxidative stress physiology, an illustration of hypothesized associations between testosterone as a measure of reproductive effort, aging, and oxidative stress responses in adult males is presented. Solid arrows indicate a stimulatory/positive influence. Dashed arrows indicate an inhibitory/negative influence.

H4. *Age will be associated with greater 8-OHdG and Cu/Zn SOD levels due to accumulated oxidative stress over time.*

1.4 | The Shuar

The Shuar are an Indigenous population living in the Amazonian regions of Ecuador and Peru. Historically, the Shuar have subsisted on a mix of foraging for forest and river food products as well as horticulture (Rubenstein 2001). The common diet of the Shuar living in forest communities consists predominantly of manioc and plantains, with occasional fish and game meat. The communities participating in this study were from the Upano Valley region of Morona Santiago province in southeastern Ecuador. This study is part of the Shuar Health and Life History Project (SHLHP) that was initiated in 2005 (Snodgrass et al. in review). SHLHP researchers have conducted numerous studies on Shuar health and behavior, including the effects of integration into Ecuadorian market economies (Blackwell et al. 2009; Liebert et al. 2013; Liebert et al. 2014; Sugiyama et al. 2016), child growth and development (Amir et al. 2016; Amir et al. 2020; Urlacher et al. 2016; Urlacher et al. 2018; Urlacher et al. 2019), immune function (Blackwell et al. 2011; Blackwell et al. 2010; Cepon-Robins et al. 2019; Liebert et al. 2018; Mcdade et al. 2012), bone health (Madimenos et al. 2020; Madimenos et al. 2011), and cortisol physiology (Liebert et al. 2025). For a comprehensive list of studies, see https://www.shuarproject.org/.

The rationales for conducting this investigation among adult Shuar males are first, testosterone levels in men living in industrialized societies are extremely variable, most likely due to lifestyle conditions such as high caloric availability, sedentism, and a low pathogenic environment (Bribiescas 2001; Ellison et al. 2002; Harman et al. 2001). Human males from communities who engage in foraging and horticulture or live under ecological and lifestyle conditions that were common throughout human evolution tend to exhibit lower testosterone levels and less variation (Bentley et al. 1993; Bribiescas 1996; Christiansen 1991; Ellison and Panter-Brick 1996; Trumble et al. 2016; Trumble et al. 2013). Testosterone can also vary significantly in association with age in adult males living in developed areas, whereas age has a modest effect on testosterone in non-industrialized communities (Bribiescas 2005; Ellison et al. 2002). In addition, antioxidant supplements and other agents that can potentially affect biomarkers of oxidative stress are not used in many populations such as the Shuar. Finally, based on a small subset of Shuar males, total energy expenditure (TEE) and water throughput measures were high compared to other populations, making them potentially informative of the effects of high metabolic costs (Christopher et al. 2019).

2 | Methods

Healthy adult Shuar males (> 18 years age) were recruited opportunistically in 2012 and 2013 from several remote Shuar communities. Participants were provided with sterile containers and instructed to collect first morning urine samples. The research protocol was approved by the University of Oregon and Yale University Institutional Review Boards (University of Oregon Protocol # 09012010.010; Yale Protocol # 1106008609) as well as the Federación Interprovincial de Centros Shuar (FICSH), the representative governing body for the participating Shuar communities.

Sample storage, handling, and transport is especially vital for maintaining the viability of 8-OHdG and Cu/Zn-SOD in urine (Matsumoto et al. 2008). Sample storage and handling of urine in the field was as follows. Morning urine samples were collected from adult Shuar males in sterile containers between 07:00 and 09:00. On the same day of collection, samples were assessed for specific gravity (SG) (Atago 4410 PAL-10S digital pocket refractometer [range: 1.000–1.060]), aliquoted into 1.0 mL polystyrene tubes, and immediately frozen at −20°C on the same day in portable freezers while in Shuar communities. SG has been shown to be preferable to creatinine corrections due to low creatinine values in non-industrialized communities (Miller et al. 2004).

Temperature was monitored using a wireless Bluetooth thermometer to avoid opening freezers. When electricity was not available, portable freezers were powered by portable solar charged batteries. Ground transportation from Shuar communities to plug-in electric commercial freezers in Sucúa, Ecuador, took approximately 10–12 h during which ice packs were used to deter thawing. During the single bus transfer, samples were stored in a local −20°C freezer. Visual inspection of samples upon arrival to −20°C freezers in Sucúa confirmed that the samples remained frozen. Similar protocols were used during ground transportation from Sucúa to Quito, Ecuador. Samples were commercially shipped on dry ice from Quito, Ecuador, to the Yale University Reproductive Ecology Laboratory (YREL) in New Haven, Connecticut, USA. Dry ice was replenished by the shipping company during airport transfers. Visual inspection of samples upon arrival to the YREL confirmed that the samples remained frozen. Urine samples were stored at −80°C until 2014–2015 before being assayed.

Testosterone (Arbor Assay, Ann Arbor, MI, kit # K032-H1, range 0–10 ng/mL), 8-OhDG (Enzo Life Sciences, Farmingdale, NY, kit # ADI-EKS-350, standard curve range 0.94–60 ng/mL, sensitivity 0.59 ng/mL), and Cu/Zn-SOD (Enzo Life Sciences, Farmingdale, NY, kit# ALX-850-033-KI01, range 0.08 to 5 ng/mL, sensitivity 0.04 ng/mL) levels were measured using an enzyme immunoassay (EIA) in the YREL. All testosterone assays were conducted by the same co-author (DA). Four 8-OHdG and Cu/Zn SOD assays were all run by the same co-author (AS). Assays were run according to the manufacturer's specifications with no modification. 8-OHdG was assayed after a 1:20 dilution in assay buffer and Cu/Zn SOD after a 1:10 dilution as recommended by the manufacturer. All samples were run in duplicate with mean values used for analysis. Interassay quality controls (QC) were as follows: testosterone—high 9.3 ng/mL, cv mean = 4.7%, low = 3.4 ng/mL, cv mean = 6.4%; 8-OHdG—high mean = 3.9 ng/mL, cv = 5.2%, low 2.2 ng/mL, cv = 22.3%; Cu/Zn SOD—high 0.33 ng/mL, cv = 14.0% and low 0.20 ng/mL, cv = 6.4%. Intra-assay variation as calculated by the means of the coefficients of variation (CVs) of sample duplicates was 10.1% testosterone, 10.2% 8-OHdG; 12.5% Cu/Zn SOD. Samples that read beyond the upper or lower range of assay detectability or exhibited intra-assay variability between duplicates exceeding 30% were excluded from analysis. While a 30% duplicate variability cut-off is acceptable, it is on the high end compared to common cut-offs of 15%. Intra-assay variability is often amplified when sample values are at the lower end of the standard curve, as is the case with this investigation. Blanks for all assays were indistinguishable from zero.

Testosterone, 8-OHdG, and Cu/Zn SOD results were corrected for SG according to Miller et al. (2004) as well as the dilution factor for Cu/Zn SOD. Target SG (1.01) was determined using the total sample mean SG.

$$\frac{\text{Testosterone, 8-OHdG, or Zn / Cu SOD concentration}_{\text{sample}}\,\text{ng / ml}^{*}\ (1.01-1)}{\left(\text{SG}_{\text{sample}}\,\text{ng/ml}-1\right)}$$

A read failure occurred in the QC cells of one of four Cu/Zn SOD assays. Standard curve performance of the Cu/Zn-SOD assay without QC data was according to the manufacturer's specifications ($r = > 0.999$). Due to the unique nature of the data, we chose to include the data from the assay lacking high- and low-QC in our analysis and use the remaining three Cu/Zn SOD assays for QC calculation. 8-OHdG and Cu/Zn-SOD values were calculated using SoftMax Pro 5.0 for Macintosh (Molecular Devices, San Jose, CA, USA). Testosterone values were calculated using the Arbor Assay testosterone protocol for Kit K032-H1 on MyAssays.com web-based software. Height (cm) (Seca, Chino, CA) weight (kg), and fat percentage (Tanita, BC-534, Arlington Heights, IL) were recorded to the nearest 0.1 at the time of urine sample return. Body mass index (BMI) was calculated as kg/m^2.

Linear regression was used to determine associations between 8-OHdG as the dependent variable and testosterone and Cu/Zn SOD as independent variables. The same associations were assessed with Cu/Zn SOD as the dependent variable. Additional multiple linear regression models were developed with 8-OHdG and Cu/Zn SOD as the dependent variables and testosterone, age, and anthropometric variables as independent variables. Models were created to test the effects of all independent variables on the dependent variables, 8-OHdG and Cu/Zn SOD. The most significant model was created for each dependent variable by eliminating independent variables that exhibited significant multicollinearity. Variance inflation factors (VIF) were calculated to quantify multicollinearity and identify independent variables subject to removal from the model. Variables with VIF > 5.0 were subject to removal from the model to mitigate the effects of multicollinearity (Vittinghoff 2005) and to identify the most plausible models based on corrected Akaike Information Criterion (AICc) values. Post hoc power analyses were conducted using G*Power 3.1.9.6 for Mac OS (Faul et al. 2009; Faul et al. 2007).

3 | Results

Descriptive data are presented in Table 1. There was no significant linear correlation between testosterone and 8-OHdG [$r^2 = 0.01$, $p = 0.61$, $n = 29$], testosterone and Cu/Zn SOD [$r^2 = 0.01$, $p = 0.68$, $n = 17$], or Cu/Zn SOD with 8-OHdG [$r^2 = 0.02$, $p = 0.53$, $n = 27$]. Post hoc power analysis of these regressions revealed a 1-beta of less than 0.10.

Models to account for 8-OHdG variation are presented in Table 2. Total Model (a) includes all variables, including those with high VIF values indicating high collinearity. Model (b) includes stepwise removal of variables from model (a) with VIF values greater than 5.0, as well as variables starting from the least significant (based on the p value) resulting in the most parsimonious model based on a significant R^2 and the lowest AICc value. Model (b) also accounts for significant linear regression associations between age/fat percentage ($r^2 = 0.024$, $p = < 0.0001$) and weight/fat percentage ($r^2 = 0.43$, $p < 0.0001$).

Results of Cu/Zn SOD models are presented in Table 3 using the same methods used for the 8-OHdG models.

Post hoc analysis of individual linear regression correlations of anthropometric measures with 8-OHdG, Cu/Zn SOD, or testosterone did not yield significant results (Table 4).

4 | Discussion

Our hypotheses that testosterone would be associated with measures of greater oxidative stress as a measure of the cost of reproduction in adult Shuar males was largely not supported. Testosterone was not individually associated with 8-OHdG (H1), Cu/Zn SOD levels (H2), or age (H4). Testosterone was, however, a variable in the most significant multivariate linear models that accounted for 8-OHdG variation. "Age", Cu/Zn SOD, and "testosterone" together were significant contributors to the most significant multivariate model for 8-OHdG, suggesting an association between oxidative stress protective agents, the cumulative influence of aging, and perhaps testosterone. Anthropometric measures were not associated with

TABLE 1 | Descriptive data of all participants. Unequal sample sizes are due to missing data from some participants because of unavailability during field data collection. Some testosterone, Cu/Zn SOD, and 8-OHdG results were excluded due to being outside the range of assay detectability or high duplicate CVs. Testosterone, 8-OHdG, and Cu/Zn SOD results are corrected for SG and assay dilution factors. SG measures include participants with unreported OS biomarker values due to being below the range of detection or high duplicate CVs, hence the dissimilarity with other data sample sizes. Given the sparseness of basic physiological data such as SG from Amazonian populations, we chose to include this data.

	N	Mean	Median	SD	Range
Age (years)	78	38.99	37.50	13.66	17–79
Height (cm)	68	161.24	162.00	5.52	148.9–173.8
Weight (kg)	68	66.44	65.65	8.81	51.2–88.8
BMI (kg/m^2)	68	25.48	25.19	2.40	19.7–32.6
Body Fat %	65	19.52	18.40	4.48	10.4–30.5
Testosterone (ng/mL)	53	5.23	5.01	2.82	0.33–11.01
8-OHdG (ng/mL)	50	55.76	52.77	23.61	13.37–121.00
Cu/Zn SOD (ng/mL)	42	2.96	2.01	2.88	0.49–14.1
Specific Gravity (SG)	94	1.01	1.01	0.01	1.00–1.03

TABLE 2 | Multiple linear regression models with 8-OHdG as the dependent variable. Bold values indicate high VIF values or results where $p < 0.05$.

	STD estimate	STD error	VIF	Adjusted R^2	*p*	AICc
8-OHdG						
a. Total model				0.51	0.10	153.19
Age	1.62	0.57	2.93		0.03	
Cu/Zn SOD	17.19	6.83	1.31		0.05	
Weight	6.36	3.83	**18.72**		0.15	
Fat percentage	−6.00	4.54	**8.90**		0.23	
Testosterone	3.80	3.19	2.35		0.28	
Height	−4.54	4.48	**18.04**		0.35	
Intercept	377.65	574.68	—		—	
b. Most significant model				**0.43**	**0.04**	**126.89**
Cu/Zn SOD	18.47	6.83	1.13		**0.02**	
Age	1.05	0.39	1.22		**0.03**	
Testosterone	0.22	2.37	1.12		0.93	
Intercept	−23.87	22.04	—		0.31	

8-OHdG, testosterone, or Cu/Zn SOD and did not improve multivariate models. Finally, 8-OHdG was not associated with Cu/Zn SOD.

The absence of stronger associations between testosterone and oxidative stress contrasts with results from non-human animal models that exhibit positive associations between testosterone and oxidative stress. Metabolic investment in sexually dimorphic plumage and behavioral displays in avian models may contribute to this difference (Alonso-Alvarez et al. 2006; Alonso-Alvarez et al. 2004; Alonso-Alvarez et al. 2007). Moreover, the present results differ from significant associations detected between the same measures of oxidative stress and reproductive effort in adult human females; these latter studies on females were conducted in the same laboratory (YREL) and employed the same oxidative stress measures and assay methods (Sancilio et al. 2021; Ziomkiewicz et al. 2016).

It may be that the metabolic costs of testosterone are insufficient in this population to generate a detectable increase in oxidative stress, or the research methods, including sample sizes, were unable to detect an association. In addition, unlike human females, much of the metabolic cost of reproductive effort in human males is extended over a lifetime in the form of sexually dimorphic tissue, contrasting the significant acute reproductive metabolic investment of gestation and lactation

TABLE 3 | Multiple linear regression models with Cu/Zn SOD as the dependent variable. Bold values indicate high VIF values (> 5.0) and *p* values less than 0.05.

	Estimate	STD error	VIF	Adjusted R^2	*p*	AICc
Cu/Zn SOD				0.63	0.27	70.58
a. Total model						
8-OHdG	0.03	0.01	1.99		**0.05**	
Age	−0.05	0.03	4.31		0.11	
Testosterone	−0.11	0.14	2.62		0.45	
Weight	−0.11	0.19	**25.79**		0.57	
Fat percentage	0.09	0.21	**11.11**		0.67	
Height	0.02	0.20	**21.08**		0.91	
Intercept	4.44	24.74	—		0.86	
b. Most significant model				0.35	0.08	40.64
8-OHdG	0.02	0.01	1.24		**0.02**	
Age	−0.04	0.01	1.32		**0.04**	
Testosterone	0.03	0.09	1.11		0.73	
Intercept	1.76	0.62			**0.02**	

TABLE 4 | Linear regression analyses of 8-OHdG, Cu/Zn SOD, and testosterone with age and anthropometric measures.

	8-OHdG			Cu/Zn SOD			Testosterone		
	N	r^2	*p*	*N*	r^2	*p*	*N*	r^2	*p*
Age	45	0.02	0.38	30	< 0.01	0.99	48	< 0.01	0.87
Height	42	0.06	0.10	27	< 0.01	0.88	43	< 0.01	0.78
Weight	42	0.01	0.45	27	0.03	0.37	43	< 0.01	0.99
BMI	42	< 0.01	0.86	27	0.09	0.13	43	< 0.01	0.87
Fat percentage	41	0.02	0.39	26	0.07	0.20	42	0.02	0.42

in females (Ellison and Bribiescas 2018). The difference in the intensity of reproductive effort over time between females and males may therefore contribute to the contrasting associations between reproductive effort and oxidative stress. In addition, the lack of an association between 8-OHdG and Cu/Zn SOD suggests that oxidative stress, as assessed by 8-OHdG in this population, was not sufficient to generate a protective response in the form of elevated Cu/Zn SOD. Or it may be that sample sizes were insufficient to detect a correlation due to high inter-individual variation.

Sex differences in SOD function may also be evident. In a study on the higher incidence of hypertension in African Americans, induced inflammation of human umbilical vein endothelial cells (HUVECs) resulted in significant increases in SOD activity in females regardless of race with increased exposure time to TNF-α-induced inflammation. However, unlike the present study which only measured Cu/Zn SOD, all three SODs were measured as a group (Mascone et al. 2023). A comparison of SOD1 and SOD2 from vastus lateralis muscle biopsies under resting and exercise conditions in adult human females and males showed no differences in either type of SOD (Galvan-Alvarez et al. 2023).

Associations between reproductive effort and oxidative stress in post-menopausal human females are evident, suggesting the cumulative effects of aging as well as lifetime gravidity and parity as a measure of reproductive effort were important contributors to oxidative stress (Sancilio et al. 2021; Ziomkiewicz et al. 2016). This partially aligns with the present study in which the most salient model for 8-OHdG variation included Cu/Zn SOD, age, and testosterone, suggesting that aging and protective measures are important in males even if they are young.

There is also a narrower range of testosterone levels and lower levels found in non-industrialized populations compared to more resourced communities. Although increased water throughput and TEE are evident among the Shuar (Christopher et al. 2019), testosterone levels may be attenuated in response to long-term energetic stresses (Bribiescas 1996, 2001). Differences in caloric supplementation and hCG administration between chronically undernourished adult human males and well-fed controls illustrate the potential impact of long-term energetic stress on hypothalamic–pituitary–testicular function and subsequent decreases in testosterone levels and variation in human adult males (Smith et al. 1975).

Previous assessments of the association between oxidative stress biomarkers and testosterone in adult human males are few and have yielded mixed results. Salivary 8-OHdG was found to be negatively associated with salivary testosterone ($r^2 = 0.04$, $p = 0.03$) in adult Japanese males exhibiting varied levels of erectile dysfunction. Six months of sildenafil treatment resulted in significant increases in salivary testosterone and decreases in salivary 8-OHdG. However, salivary measures of 8-OHdG remain to be fully validated, and their associations with intracellular and circulating measures necessitate further investigation (Yasuda et al. 2008). Testosterone, however, was not associated with testicular 8-OHdG in adult males with varicocele, an enlargement of blood vessels in the scrotum that can compromise spermatogenesis (Ishikawa et al. 2007). Collegiate wrestlers who underwent significant weight loss exhibited declines in testosterone, adiponectin, and leptin while simultaneously showing increases in 8-OHdG. However, no significant correlations were detected between testosterone and 8-OHdG (Yanagawa et al. 2010). Associations between anthropometric measures, age, and oxidative stress biomarkers are mixed in the literature, while 8-OHdG has been reported to be negatively associated with BMI (Zanolin et al. 2015).

A 6-month study of the effects of testosterone administration on oxidative stress, body composition, and other metabolic measures in older adult Danish males ($n = 38$, ages 68–78) resulted in a significant *decrease* in urinary 8-OHdG levels (Christensen et al. 2024). The authors suggest that the decrease was due to changes in body composition, specifically increases in lean body mass and decreases in adiposity. The authors acknowledge that their results differed from similar studies that did not observe a decrease in oxidative stress in response to testosterone administration (Mancini et al. 2008; Unluhizarci et al. 2020).

In contrast to the present investigation, most studies of testosterone and oxidative stress in human males focused on older (> 60 years) and hypogonadal adult human males from industrialized populations. Aging and hypogonadism commonly result in lower metabolic costs that may attenuate the immediate effects of oxidative stress (Fukagawa et al. 1990; Leonard et al. 2014; Pivonello et al. 2019; Pontzer et al. 2021). Fatherhood may also play a role since testosterone is widely responsive and associated with paternal investment across a wide range of ecologies and lifestyles (Gettler et al. 2011; Gray 2003; Gray et al. 2007; Gray et al. 2006). In other forager communities such as the Hadza, paternal engagement with their children is particularly associated with lower testosterone (Muller et al. 2009). The variability in the association between testosterone and oxidative stress in adult human males illustrates the importance of the energetic status of participants, immunological stress, paternal status, ecology, activity, diet, and lifestyle, especially in regard to detecting evidence of life history trade-offs (Bribiescas 2001; Bribiescas and Ellison 2007; Jasienska 2009; Muehlenbein and Bribiescas 2005; Speakman and Garratt 2014).

4.1 | Study Limitations

The limitations of this investigation include the need for more information on activity, immunological challenges, and other sources of testosterone and oxidative stress variation as well as sample size. High physical activity contributes to oxidative stress and may increase testosterone levels. Among the Tsimane of Bolivia, testosterone levels were higher in men engaging in greater levels of physical labor (Trumble et al. 2013). The present study measured whole body oxidative stress as determined by clearance of repaired guanine base pairs, 8-OHdG, and Cu/Zn SOD. Oxidative stress can vary between specific tissues and urinary measurements of 8-OHdG, or any other oxidative stress biomarker. For example, increased 8-OHdG expression in the testes was associated with lower spermatogenesis in infertile men with varicocele (Ishikawa et al. 2007). Cu/Zn SOD levels can also differ between human tissues (Kurobe et al. 1990). It is therefore challenging to assign physiological significance to specific tissues and organs from whole body oxidative stress assessments.

8-OHdG and Cu/Zn SOD are important biomarkers of oxidative stress, but others merit attention, including isoprostanes and lipid peroxidative factors such as H_2O_2, which can also be determined by measures of thiobarbituric acid reactive substances (TBARS). Inter-and intra-cellular levels of H_2O_2 and other peroxidative factors are central for the oxidation of lipids and adipose tissue. Research resources and logistical limitations constrained the measures conducted in this study, but other oxidative stress biomarkers may add clarity to future investigations. Direct measures of reactive oxidative species (e.g., OH^-, free radicals, H_2O_2) would be useful but challenging under remote field conditions.

Smoking is another source of 8-OHdG variation with higher levels exhibited by smokers (Kopa-Stojak and Pawliczak 2024). While tobacco or other substance smoking is rare among the Shuar, we were unable to assess the impact of this potential variable. We were also unable to assess the contribution of potential environmental carcinogens. While this is unlikely due to the remoteness of many Shuar communities, increased engagement with market-based economies, modern farming practices, and potential carcinogens in local rivers may play an increasing role in promoting oxidative stress in these communities (Tope and Panemangalore 2007; Webb et al. 2018).

Urinary hormone measures have many advantages such as ease of sample collection, smoothing of pulsatility variation, and valid/robust assay methodologies (Urlacher et al. 2022). However, urinary measures of any biomarker may indicate clearance variation that may not align with circulating levels. It is important for researchers to be aware of all potential sources of variation. Urinary measures of testosterone under conditions of obesity, hypogonadism, or acute physical activity may reflect increased hormone clearance independent of circulating levels (Brocks et al. 1996; Cadoux-Hudson et al. 1985; Klibanski et al. 1981). There is also evidence of population variation in clearance patterns (Santner et al. 1998; Wang et al. 2004).

A more informative test of the potential role of oxidative stress as a cost of reproductive effort may be a comparison of a broader range of oxidative stress measures in age-controlled adult males, non-reproductive adult females (non-lactating, non-pregnant), and adult females investing metabolically in reproductive effort (lactating or pregnant) between resourced and less resourced communities. Finally, the present investigation included relatively young adult males (mean age 38.99). Given the positive associations between reproductive effort and oxidative stress in post-menopausal human females, and the modest association of Shuar 8-OhdG with Cu/Zn SOD and age, it may be that the effects of oxidative stress are not evident until later in life, perhaps as a form of antagonistic pleiotropy. That is, the costs of reproduction are not evident until reproductive value is negligible or extremely low.

In conclusion, this investigation is among the first to test for an association between testosterone and important measures of oxidative stress in adult males in an Indigenous foraging/horticulturalist population undergoing integration into wage labor and market economies. The results of this investigation provide modest support for the presence of a trade-off between reproductive effort and maintenance in Shuar males, although caution is warranted, and more research is necessary due to small sample sizes and the logistical challenges of measuring biomarkers of oxidative stress under remote field conditions. Nonetheless, this investigation satisfies the core conditions for observing such a trade-off including: (1) the measurement of well-established biomarkers of reproductive effort; (2) the measurement of well-established biomarkers of maintenance, morbidity, and/or mortality; and (3) a population that exhibits compelling evidence of energetic costs that are likely to elicit allocation mechanisms between competing needs (Bribiescas and Ellison 2007; Burger et al. 2010; Ellison 2003; Jasienska 2009; Speakman and Garratt 2014; Stearns 1989). This research aims to offer insights into the evolution of human male reproductive strategies, health, and aging. Future research should include direct measures of reactive oxidative species, broader measures of hormones, other measures of reproductive effort (i.e., gravity, parity), and the incorporation of other members of the community, specifically adult females, juveniles, and the elderly.

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Data Availability Statement

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

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