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Men's oxidative stress, fluctuating asymmetry and physical attractiveness

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Aerobic organisms naturally create reactive oxygen species (ROS) as by-products of energy production. These substances can damage DNA and tissue, and probably are major causes of mutation, ageing and a host of diseases. Oxidative stress occurs when an organism produces an overabundance of ROS relative to ROS-neutralizing antioxidants. In this study, we examined the hypotheses that individual differences in oxidative stress are associated with fluctuating asymmetry and with perceived mate quality. We measured urinary biomarkers of oxidative stress (8-hydroxy-2′-deoxyguanosine (8-OHdG) and malondialdehyde (MDA)) in a sample of healthy, young men and tested their association with fluctuating asymmetry and physical attractiveness. A composite measure of oxidative stress correlated positively with FA and negatively with physical attractiveness (with exposure to toxins, smoking, and their interaction statistically controlled for). Follow-up analyses yielded associations of oxidative stress with both healthy and masculine appearance. No association was mediated by cortisol or testosterone. These findings may yield insights into previously unidentified factors that affect the development of phenotypic features under sexual selection and contribute to the shape of human life histories, and have potential implications for other species as well.
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Reactive Oxygen Species (ROS) and Oxidative Stress

The production of energy via cellular respiration entails, as a by-product, the creation of reactive oxygen species (ROS), oxygen-containing molecules or atoms that possess an unpaired electron (radicals). The electron transport chain involved in oxidative phosphorylation generally fuels energy metabolism efficiently, but at particular steps electrons are prematurely leaked to oxygen to produce radicals, most notably superoxide anion. Through subsequent reactions, superoxide anion can yield other radicals such as hydrogen peroxide, hydroxyl radical and peroxynitrate (see Valco et al. 2007).

ROS are unstable. They react with other substances, including lipids, proteins and DNA. Ageing itself may largely result from oxidative cellular damage (e.g. Beckman & Ames 1998). And oxidative damage to DNA appears to be the overwhelmingly primary cause of mutation (Denver et al. 2009). Accordingly, aerobic organisms possess special adaptations to obviate harm due to ROS: enzymes (e.g. superoxide dismutase, catalase) that convert ROS into stable, harmless molecules (e.g. catalase converts hydrogen peroxide into water and oxygen); use of antioxidants acquired in diet (e.g. tocopherol); repair of some ROS-induced damage (e.g. Valco et al. 2007).

An organism is said to experience oxidative stress when ROS production exceeds antioxidant capacity, yielding susceptibility to oxidative damage. In addition to causing senescence and mutation, oxidative stress contributes to the pathogenesis of various diseases (e.g. cancers, neurodegenerative disorders, diabetes; Valco et al. 2007). ROS are also involved in adaptive processes (produced by macrophages to kill pathogens; at low levels, co-opted to serve roles in cell signalling; Valco et al. 2007).

ROS within Evolutionary Biology

Recently, ROS and oxidative stress have received attention from evolutionary biologists (e.g. Dowling & Simmons 2009). ROS are critical to evolutionary theory for at least two reasons. First, they importantly inform life history theory. This theory refers to broad categories of resource allocation (e.g. somatic and reproductive effort), often without fleshing out their precise natures. A significant ongoing task is explicit specification of subcomponents of allocation. Defence against oxidative stress through production of antioxidants and repair processes may constitute important allocations to somatic effort, and may be particularly relevant for

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species with low extrinsic mortality. Moreover, because ROS are intrinsic costs of energy production itself, oxidative stress is a constraint on other expenditures.

As limited energy budgets force a trade-off between somatic effort and reproductive effort, one major cost of reproduction may be the risk of oxidative damage (Alonso-Alvarez et al. 2007; Dowling & Simmons 2009). Consistent with this view, manipulated increases in reproductive effort (e.g. stimulating female egg production, injecting male birds with testosterone) increase oxidative stress (e.g. Wang et al. 2001; Alonso-Alvarez et al. 2004). When conceptualized within a life history framework, ROS and oxidative stress need not be dysfunctional outcomes; they arise as natural by-products (albeit harmful ones). When optimally allocating effort, organisms will not perfectly resist the harmful effects of ROS (e.g. Kirkwood 1977).

Second, ROS may inform theories that assume that individuals vary in 'quality' or 'condition'. As Rowe & Houle (1996) noted, condition often refers to 'the pool from which resources are allocated' (page 1416). An individual's quality or condition, however, may be a function of not merely a resource pool, but also the efficiency with which resources are converted into fitness enhancement. As noted above, ROS are intrinsic costs of energy production itself. The net gain of energy production is a function of the fitness enhancement of activities that energy permits minus the costs associated with ROS. These costs may vary, for example, as a function of the rates at which superoxide anion is created or converted into more damaging radicals, themselves functions of mutations, nutrition and other factors (e.g. Murphy 2009). The ROS-induced cost per unit energy production is a component of the efficiency of metabolism (i.e. net gain per unit of energy production). Variation between individuals in this form of efficiency may constitute an important component of individual differences in quality.

Much sexual selection theory is presently founded on life history strategies in concert with individual differences in condition (e.g. Kokko et al. 2003). Optimal allocation strategies may vary contingent upon condition. Individuals in good condition may benefit through greater allocation to particular forms of reproductive effort (e.g. sexual signals, male–male competition), leading them to covary with condition (e.g. Getty 2002; Kokko et al. 2003). If the efficiency of energetic production contributes to condition, individuals allocating relatively great effort to sexual signalling or male–male competition may pay lower costs in the form of ROS for such allocations than others (e.g. Mougeot et al. 2009; also von Schantz et al. 1999; Alonso-Alvarez et al. 2007).

The Current Study: Primary Aims

The current study applied an evolutionary perspective on ROS to an understanding of variation between men, focusing in particular, on two primary hypotheses: (1) levels of oxidative stress are positively associated with men's fluctuating asymmetry (FA); (2) levels of oxidative stress are negatively associated with men's physical attractiveness.

Oxidative stress and FA

FA is the primary measure of developmental instability (DI), the imprecise expression of developmental design owing to perturbations of development. Asymmetries in bilateral features symmetrical at the population level are attributed to multiple, largely independent errors in developmental processes. Although some studies have identified risks for DI (e.g. toxins: Eeva et al. 2000; inbreeding: Carter et al. 2009), the precise processes through which asymmetrical growth occurs largely remain in a developmental black box (see Polak 2003). Oxidative stress may be one process through which asymmetries are created. ROS-induced damage to DNA or cell membranes may disrupt cell replication. We therefore predicted that individual differences in susceptibility to oxidative stress should be associated with FA. We examined the association between men's FA and biomarkers of oxidative stress. As FA may be associated with birth stresses (Livshits et al. 1988), we also examined associations with oxidative stress with a composite of various births stresses (e.g. early or late birth, low birth weight, delayed release from hospital).

More generally, we note, there is a relative paucity of research revealing why developmental instability might be associated with fitness, specifically in humans. A recent meta-analysis of studies on human FA reveals, on average, modest yet robust effects on a variety of fitness-related outcomes, such as disease, poor fetal outcomes and psychological dysfunction (S. Van Dongen & S. W. Gangestad, unpublished data). Yet precisely how and why FA becomes linked to a wide variety of diseases and dysfunction remains unknown. Once again, oxidative stress may play a role (e.g. oxidative stress may give rise to both developmental instability and disease proneness).

Oxidative stress and physical attractiveness

Physical attraction of females to males may be thought of as an aggregate effect on female choice of male physical features. If females benefit (directly or indirectly) by mating with males with relatively low susceptibility to oxidative stress, they may evolve to be attracted to features associated with low oxidative stress, a hypothesis provisionally assessed by examining the association between male oxidative stress and physical attractiveness. As secondary aims, we explored two candidate components of attractiveness: the healthiness and masculinity of a man's appearance (e.g. Rhodes et al. 2007).

We restricted our examination of associations to men because (1) women's FA may vary with changing hormone levels across the menstrual cycle (e.g. Manning et al. 1996) and (2) female allocation to sexual signalling, although present, typically operates under added constraints (e.g. Chenoweth et al. 2006).

We tested predictions by measuring two urinary biomarkers of oxidative stress: 8-hydroxy-2′-deoxyguanosine (8-OHdG), the most validated urinary biomarker of oxidative DNA damage (Mayne 2003; Wu et al. 2004; Tamura et al. 2006), and malondialdehyde (MDA), a common biomarker of lipid peroxidation (e.g. Mayne 2003). Technically, 8-OHdG is a by-product of the repair of DNA with damaged guanosine sites, typically thought to be due to oxidative damage. MDA is a by-product of lipid peroxidation per se (see Mayne 2003). Following standard terminology, we refer to both as markers of oxidative stress. Most prior research has examined roles of oxidative stress in disease, not in healthy individuals, and both measures have been successfully and commonly used in this regard. 8-OHdG, for instance, has been found to be elevated in individuals with diabetes, cancer and atherosclerosis (reviewed in: Wu et al. 2004; Valavanidis et al. 2009). Some research shows that elevated 8-OHdG is a risk factor for these diseases, not merely an outcome. For instance, prediabetic individuals, as well as those with diabetes, have elevated levels of urinary 8-OHdG compared to controls (Al-Aubaidy & Jelinek 2010). Elevated maternal 8-OHdG levels early in pregnancy predict low birth weight and short gestations of infants (Stein et al. 2008). Similarly, MDA has been found to be elevated in both mothers and infants with small-for-gestational-age births (Gveric-Ahmetasevic et al. 2009). In addition, for instance, urinary MDA is elevated in individuals with parasitic infection (Chandramathi et al. 2009) and in individuals that experience a relapse of acute myeloid leukemia (Zhou et al. 2010), that experience cancer-prone inflammatory disease (see review by Nair et al. 2007) or that have been diagnosed with schizophrenia (see meta-analysis by Grignon & Chianetta 2007).

Current Study: Secondary Aims

In addition to assessing associations of oxidative stress biomarkers with FA and physical attractiveness, we addressed questions pertaining to possible roles played by two steroid hormones, cortisol and testosterone. Glucocorticoids modulate energy utilization in the face of energetic stress in mammals and many other vertebrates (e.g. Sapolsky et al. 2000). In humans, they also modulate energy utilization in response to psychosocial stressors (e.g. Sapolsky 1998). Testosterone is a reproductive hormone, particularly important in males in most vertebrate species, that modulates the utilization of energy in response to demands for mating effort (Wingfield et al. 1990) or various forms of somatic effort (e.g. immune function; Muehlenbein & Bribiescas 2005). In humans and some other biparental species, it also appears to modulate energy utilization in response to parental demands (e.g. Ellison 2003).

One or both of these hormones could, in theory, mediate an association between FA or physical attractiveness and oxidative stress. A mediator is an intermediary step in a causal change. Suppose that oxidative stress is caused by the production of cortisol (or the energetic or psychosocial stressors that cause cortisol production; e.g. Epel et al. 2006). If so, and if FA or physical attractiveness causes increases in cortisol (e.g. due to the psychosocial stress associated with low mating success), cortisol could mediate an association between FA or physical attractiveness and oxidative stress. We measured levels of salivary cortisol at the same time points that we assessed oxidative stress biomarkers and examined its possible mediating role. If cortisol mediates an association between, for instance, physical attractiveness and oxidative stress, then cortisol should be associated with each variable itself. Moreover, a partial correlation between these two variables controlling for cortisol should reduce the correlation substantially (e.g. Baron & Kenny 1986). As testosterone may also lead to increased oxidative stress (e.g. Alonso-Alvarez et al. 2007; Mougeot et al. 2009), we examined its associations with oxidative stress as well.

METHODS

Participants

We recruited 98 male participants, all taking a psychology course at the University of New Mexico, for which they partially fulfilled a research requirement. Participants were confined to a relatively narrow age group (mean $\pm$ SD age $= 20.1 \pm 2.9$ years, range 18–38 years).

All procedures on participants were conducted in accord with guidelines for ethical research with human subjects, and the protocol was approved by the University of New Mexico Human Research and Review Committee (protocol 08-023).

Procedures

Participants arrived for a scheduled laboratory session. After providing informed consent, the participant was given a questionnaire to complete in a private room. Questionnaire content included a number of variables pertinent to our analyses, described below (e.g. cigarette smoking, exposure to toxins). During the session, each participant was brought to a separate laboratory room, where we measured FA and took two photographs.

Assessment of FA

We measured 10 bilateral features: ear width, ear height, wrist width, elbow width, lengths of four fingers, ankle breadth and foot breadth. The left and right sides were measured twice with precise digital calipers. To ensure that replicate measures were independent, they were separated by intervening measurements, and the measurer called out values to a recorder (discouraging memory for them). Prior work has used the same procedures (e.g. Furlow et al. 1997; Gangestad & Thornhill 1998; Thornhill & Gangestad 1999; Thornhill et al. 2003; Gangestad et al. 2005).

Composite measure of FA

Because a single trait's FA taps organism-wide developmental instability very weakly (e.g. Van Dongen 1998; Whitlock 1998; Gangestad & Thornhill 1999), we aggregated the FA of all 10 features into a composite measure. To create this measure, we (1) averaged the two right-side and the two left-side measures for each trait for each individual, (2) took the absolute difference between sides for each trait for each individual, (3) standardized each trait's FA by dividing these absolute differences by the mean trait size for the sample and (4) summed these standardized values across all 10 traits for each individual. To ease interpretation, we multiplied the sum by 10. The mean composite FA for the sample of 1.70 means that, on average, across the 10 traits, the average man's asymmetry was 1.7% of the mean trait size. The SD was 0.46, the range 0.72 to 2.88. These values are similar to previous studies (e.g. Furlow et al. 1997; Gangestad & Thornhill 1998; Thornhill & Gangestad 1999; Thornhill et al. 2003; Gangestad et al. 2005).

We asked each individual whether he had broken, sprained or injured any feature measured and, if so, we substituted the mean asymmetry if asymmetry exceeded it. This procedure, which affected 3.5% of all asymmetry values, eliminates large asymmetries caused by injury.

Measurement reliabilities

The mean correlation between unsigned, absolute differences was 0.77 (range 0.72–0.89; all $P < 0.00001$) and that between signed differences was 0.88 (range 0.84–0.94; all $P < 0.00001$). The correlation between first and second measurements of composite FA was 0.81, $P < 0.00001$, similar to other studies (see list above).

Developmental repeatabilities

Signed FA due to individual differences in developmental instability and random developmental error should exhibit slight leptokurtosis, as our measures did (mean kurtosis $= 0.46$; see Gangestad & Thornhill 1999). Developmental repeatabilities, estimates of the proportion of variance in a trait's FA due to systematic individual differences, were estimated using three published methods (Van Dongen 1998; Whitlock 1998; Gangestad & Thornhill 1999). On average, across the traits, estimated repeatability was 0.092, 0.033 and 0.067 for the three methods, respectively, for a grand mean of 0.064. Gangestad et al. (2001) reported a similar value (0.078) in college men. This level of developmental repeatability is theoretically expected and typically observed empirically across a wide variety of species (Gangestad & Thornhill 1999, 2003). (For these analyses, all injured features were excluded.)

Directional asymmetries

We also examined signed right–left (R-L) differences for directional asymmetries. With no correction for multiple comparisons, the mean R-L difference deviated significantly from zero for 4 of 10 traits: ear length, elbow width, fifth finger length and ankle width. (For the first three traits, R > L; for the last one, L < R.) These differences, which were small (0.32 to 0.54 standard deviations from zero asymmetry), could be due to actual directional asymmetries or to systematic biases in measurement (e.g. the measurer not holding the calipers in a precisely mirror-imaged way in measuring the two sides). If these differences are due to differential

use of the two sides (e.g. right-handers using their right arms more), they should covary with handedness, with right-handed men, the majority of individuals (91.5% versus 8.5% left-handed men), driving the directional bias (see Van Dongen et al. 2009, for this argument). Yet none of these four signed differences (or the signed differences for the other six traits, for that matter) covaried significantly with handedness, all $P > 0.3$, and the mean point-biserial correlation between handedness and signed differences (scored in the direction expected if the right-handed majority drives the directional bias) was 0.00. The complete lack of evidence that handedness drives directional biases strongly suggests that differential use of the two sides does not explain biases (cf. Van Dongen et al. 2009; see also Furlow et al. 1997, for failure to find, in a very large sample, robust associations between handedness and these asymmetries).

Nevertheless, we also created a composite index that controlled for directional asymmetries. Specifically, FA for these four features was computed by taking the deviation from the sample-wide mean asymmetry (see Simmons et al. 2004). This index correlated 0.94 with the unadjusted composite, which means that adjustment made virtually no difference to the composite measure (due to the modest size of the directional deviations). Furthermore, the two measures yielded near-identical results. For the sake of simplicity, we report the results for the unadjusted measure and note that, for every significant effect that we report for this composite measure, we also found a significant association for the composite measure adjusting for directional bias (full results available from the authors).

Assessment of physical attractiveness

Each participant stood against a blank wall. With a digital camera, we took two photographs: one of each male's full body, and one of each male's face alone. Participants were asked to look straight ahead, with a neutral expression. We requested written consent to have photographs rated for attractiveness; 89 men consented, 9 declined. Five women unfamiliar with the participants rated each man's attractiveness on a 1 to 10 (least to most attractive) scale (composite $\alpha = 0.80$). As our primary aim was to examine associations with overall attractiveness, raters viewed both photographs of a man when making their assessment.

Each woman also rated the healthiness and masculinity of each man's appearance (composite $\alpha = 0.82$ and 0.79, respectively). (Although our number of raters was modest, the reliabilities of their ratings, averaging over 0.8, indicated that they agreed with each other so strongly that increasing the number of raters would not have made a substantial difference.)

Assessment of urinary biomarkers of oxidative stress

During the session, each man was asked to provide a 10 ml urine sample, immediately frozen at −20 °C. Each participant was also given a test-tube in which to collect a first-of-the-day urine sample 7 days after the initial session and either bring the sample to us immediately after collecting it, or freeze it until it could be brought, most all the same or next day. Upon delivery, we froze each sample at −20 °C. Although in principle biomarker levels could have changed if not immediately frozen at −20 °C, in fact, levels of 8-OHdG, at least, are stable even if kept at room temperature for 24 h (Matsumoto et al. 2008).

8-OHdG was assayed in duplicate using ELISA kits manufactured by the Japan Institute for the Control of Aging (8-OHdG Check, distributed in the U.S. by Genox, Baltimore, MD; e.g. Tamura et al. 2006). MDA was assayed colorimetrically using an assay distributed by Northwest Life Sciences Specialties (Vancouver, VA, U.S.A.) and performed in triplicate. Levels of each biomarker were standardized against levels of creatinine quantified colorimetrically (Taussky 1954), performed in quadruplicate, with overly dilute samples excluded from further analysis (creatinine < 0.10 mg/ml, $N = 5$). All assays were performed in the University of New Mexico Hominoid Reproductive Ecology Laboratory. We found no associations (linear or curvilinear) between biomarker levels and time since waking and, hence, did not adjust for time of collection.

In Table 1, we list the means and standard deviations of measures of 8-OHdG and MDA in our participants. Sensitivity of the 8-OHdG assay was 0.5 ng/ml, with an interassay coefficient of variation (CV) of 11%. The average CV of sample replicates was 6.6%. Sensitivity of the MDA assay was 100 nmol/ml, and interassay CV was 17%. CVs for replicate MDA determinations averaged 29%. However, this was influenced by the fact that many subjects had very low levels of MDA, for which high CVs reflected small differences in measurement relative to the range of values measured in this study. Among samples with high MDA (above average), the CVs of replicates averaged 14%.

In theory, an aggregate measure incorporating both biomarkers (each assessing different outcomes of oxidative stress) should more validly assess levels of oxidative stress than a single biomarker. To most powerfully test our prediction, then, we created an aggregate measure of oxidative stress by z scoring each biomarker at each time point and averaging, for each individual, the four z scores. For these analyses, we included only men who provided both samples and on whom we had complete information on oxidative stress ($N = 71$). In follow-up analyses on the first sample, we included all men on whom we had valid values for both biomarkers ($N = 94$).

Assessment of salivary cortisol and testosterone

We also asked participants to provide us with a sample of saliva (~10 ml) at each of two periods of urine collection. Saliva samples were transferred from a cup to a test-tube with a pipette, then capped and frozen at −20 °C. Frozen samples were thawed, mixed by vortexing, then centrifuged for 15 min before being used for cortisol and testosterone assays.

Salivary testosterone concentrations were determined by radioimmunoassay (RIA) (DSL-4100, Diagnostic Systems Laboratories, Webster, TX, U.S.A.) following the protocol for saliva developed by Granger et al. (1999) and using kit standards diluted to a range of 2–500 pg/ml. Interassay CV was 10.7% and the intra-assay CV for duplicate determinations averaged 9.4%.

Salivary cortisol concentrations were also determined by RIA, using a commercial kit (DSL-2000). Kit controls were diluted to 0.15–18.0 ng/ml, and both antibody and tracer were used in 1:2 dilutions and 150 µl aliquots. As with the testosterone RIA, we used 200 µl of phosphate-buffered saline to buffer all tubes, and applied an overnight incubation procedure, 500 µl aliquot of second antibody, and extended (45–60 min) centrifugation to maximize separation of bound and unbound fractions. Interassay CV was 5.4% and intra-assay CV for duplicate determinations averaged 6.4%.

As both testosterone and cortisol levels are subject to diurnal variation, we controlled for duration passed since a participant

Table 1
Means and standard deviations of oxidative stress biomarkers

Biomarker	Sample	Mean	SD	*N*
8-OHdG	Sample 1	975.8	407.7	94
	Sample 2	1066.0	486.6	75
MDA	Sample 1	353.4	194.9	94
	Sample 2	375.4	175.2	74

8-OHdG = 8-hydroxy-2′-deoxyguanosine; MDA = malondialdehyde. Urinary 8-OHdG measured in units of ng/mg of creatinine. Urinary MDA measured in units of nmol/mg of creatinine.

awakened. In these analyses, we entered polynomial terms of time awake and we stopped when the last term entered was not significant. Only the linear effect of time awake significantly predicted testosterone levels. Both the linear and quadratic effects of time awake predicted cortisol levels. Residual values of each hormone were calculated and used in all analyses.

Assessment of potential confounds and other correlates of oxidative stress

Oxidative stress may be modestly affected by regular exposure to toxins (e.g. heavy metals, fumes; e.g. Kimura et al. 2006; Pilger & Rudiger 2006) and cigarette smoking (e.g. Sakano et al. 2009). We asked each participant whether he (1) worked in a setting that regularly exposed him to environmental toxins (e.g. fumes, chemicals) and (2) currently smoked cigarettes and, if so, how many per day (with categories 0 = 0, 1 = 1–5, 2 = 6–10, 3 = 11+). Both exposure to toxins (point-biserial $r = 0.26$, $N = 71$, $P < 0.05$) and level of current smoking ($r = 0.26$, $N = 70$, $P < 0.05$) predicted oxidative stress. As we propose that associations of oxidative stress with developmental instability and physical attractiveness are attributable to long-term, stable individual differences, we controlled for these current factors affecting oxidative stress and their interaction (e.g. Chia et al. 2008) in all analyses on oxidative stress (e.g. it would perhaps be uninteresting if less attractive people were more likely to take up smoking, and as a result have higher levels of oxidative stress; controlling for current levels of smoking yields results uncontaminated by such effects). In addition to asking men about current smoking, we asked them whether they smoked regularly in the past. Past smoking did not incrementally predict oxidative stress and, therefore, we did not include it as a control variable. Age was not associated with oxidative stress in this relatively young sample ($r = 0.14$, $N = 71$, $P = 0.263$).

As dietary antioxidants (e.g. vitamins) may affect oxidative stress (e.g. Mayne 2003), we also asked participants about diet. Specifically, we summed responses to questions about how balanced they consider their diet and the extent to which they consumed healthy or unhealthy foods (measured on seven-point response scales; Cronbach's $\alpha = 0.85$). In addition, we asked men whether they took vitamin or herbal supplements. Although past research does not yield clear effects of exercise on oxidative stress (Reichhold et al. 2009), we also asked individuals to rate, on seven-point scales, the extent to which they engaged in physical activity, aerobic exercise and strength-building exercise. Cronbach's α of a composite was 0.90.

Birth complications (e.g. atypical birth weight, preterm birth, maternal hypertension) may result in stable increases in oxidative stress (e.g. Franco et al. 2007; Mohn et al. 2007; Chiavaroli et al. 2009; Nassi et al. 2009). Factors leading to birth complications may constitute individual differences in production of ROS that we speculate affect developmental instability or attractiveness. To explore associations with birth stresses, then, we inquired about nine of them: premature birth, late birth, low birth weight (<2.5 kg), very low birth weight (<1.5 kg), specialized neonatal care, postbirth hospitalization, maternal bed-rest owing to complications, maternal diabetes, maternal hypertension. To construct a composite measure, we totalled the number checked (mean ± SD = 0.63 ± 1.15, range 0–5).

Analysis

For predicted associations, we applied directed tests. As recommended (Rice & Gaines 1994), 0.04 of the total 0.05 type I error rate was allocated to the predicted direction, 0.01 to the unpredicted direction. For all other tests, we used two-tailed tests.

RESULTS

FA and Oxidative Stress

We examined the correlation between FA and oxidative stress, with toxin exposure, smoking, and their interaction partialled out. As expected, FA significantly and positively predicted oxidative stress (partial $r = 0.26$, $N = 70$, $P = 0.021$; Table 2, Fig. 1).

Physical Attractiveness and Oxidative Stress

We performed a similar analysis substituting physical attractiveness for FA. Again, as expected, physical attractiveness significantly and negatively predicted oxidative stress (partial $r = -0.30$, $N = 63$, $P = 0.012$). As physical attractiveness increased, levels of oxidative stress biomarkers decreased (Table 2, Fig. 2).

Perceived Healthiness, Perceived Masculinity and Oxidative Stress

Both healthy appearance and masculine appearance covaried significantly with attractiveness ($r = 0.84$ and 0.63, respectively, $P < 0.0001$). To explore contributions of these two components of attractiveness, we did two additional analyses, each substituting healthy or masculine appearance for attractiveness.

Both perceived healthiness and masculinity significantly predicted oxidative stress (partial $r = -0.30$ and -0.24, respectively, $N = 63$, $P < 0.05$). These effect sizes did not significantly differ ($t_{57} = 0.55$, $P = 0.579$; Table 2).

Correlations of FA with the three ratings were computed. FA significantly and negatively predicted physical attractiveness ($r = -0.22$, $N = 89$, $P = 0.025$) and masculinity ($r = -0.30$, $N = 89$, $P = 0.003$), but not healthy appearance ($r = -0.14$, $N = 89$, $P = 0.127$). These correlations did not significantly differ (all pairwise $t_{86} \leq 1.74$, $P \geq 0.085$).

Birth Complications

Birth complications predicted oxidative stress levels (partial $r = 0.25$, $N = 70$, $P = 0.026$; Table 2). Birth complications covaried with FA and masculine appearance at marginal levels of statistical robustness ($r = 0.16$, $N = 98$, $P = 0.070$; $r = -0.15$, $N = 89$, $P = 0.100$), but did not significantly predict attractiveness ($r = -0.13$, $N = 89$, $P = 0.134$) or healthy appearance ($r = -0.10$, $N = 89$, $P = 0.236$).

We followed up with examinations of specific complications, several of which significantly predicted oxidative stress: late birth ($r = 0.26$), specialized neonatal care (0.23) and postbirth

Table 2
Partial correlations with oxidative stress biomarkers‡

	FA	Physical attraction	Healthy appearance	Masculine appearance	Birth complications
Oxidative stress composite§	0.26*	−0.30*	−0.30*	−0.24*	0.25*
Subcomponents					
8-OHdG	0.24*	−0.26*	−0.27*	−0.28*	0.18†
MDA	0.16	−0.19†	−0.18†	−0.06	0.21†
Sample 1 (lab session)	0.15†	−0.23*	−0.21*	−0.12	0.29**
Sample 2 (early AM)	0.16	−0.08	−0.13	−0.23*	0.11

FA = fluctuating asymmetry; 8-OHdG = 8-hydroxy-2′-deoxyguanosine; MDA = malondialdehyde.
†$P < 0.10$; *$P < 0.05$; **$P < 0.01$.
‡ Smoking, exposure to toxins, and their interaction partialled out (see text).
§ Aggregated oxidative stress biomarkers (see text).

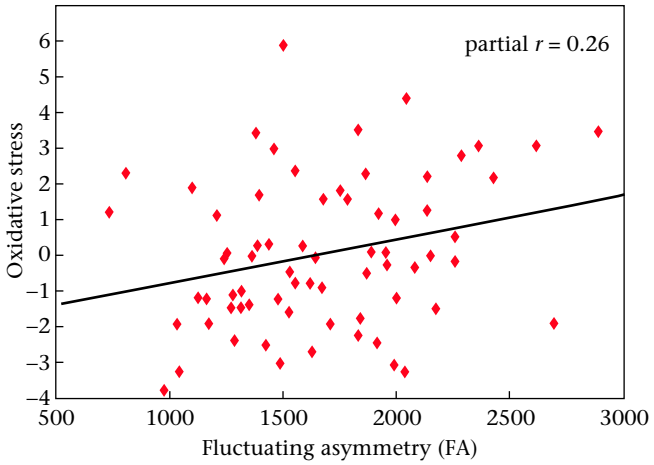

Figure 1. Scatterplot of men's fluctuating asymmetry (FA) and oxidative stress. Values are mean-centred residuals, with toxin exposure, smoking, and their interaction partialled out.

hospitalization (0.27), all $P < 0.05$. No firm conclusions can be drawn, however, about the relative strength of association between particular forms of birth complications and other complications, given the multiple comparisons that were made. That is, some complications did not outperform others in a statistically robust way.

Cortisol and Testosterone

Neither cortisol nor testosterone levels were significantly associated with the oxidative stress composite (partial $r = 0.14$, $N = 70$, $P = 0.260$; partial $r = 0.02$, $N = 70$, $P = 0.880$). Moreover, no nonzero correlation of hormone level with FA and physical attractiveness was detected (cortisol with FA and attractiveness: $r = -0.03$, $N = 98$, $P = 0.755$; $r = 0.00$, $N = 89$, $P = 0.564$; testosterone with FA and attractiveness: $r = -0.05$, $N = 98$, $P = 0.615$; $r = -0.04$, $N = 89$, $P = 0.721$; see also Peters et al. 2008).

If cortisol or testosterone mediates associations of FA and attractiveness with oxidative stress, then controlling for the mediating hormone should eliminate or attenuate the associations. Not surprisingly, in light of the above correlations, correlations of FA and physical attractiveness with the oxidative stress composite remained virtually unaltered when either hormone was partialled out (FA: partial $r = 0.25$, 0.26, $N = 70$, both $P < 0.04$; physical attractiveness: both partial $r = -0.30$, $N = 63$, $P < 0.02$). We found no evidence that either cortisol (or stressors of which it is a biomarker) or testosterone mediate relationships of FA and physical attractiveness with oxidative stress.

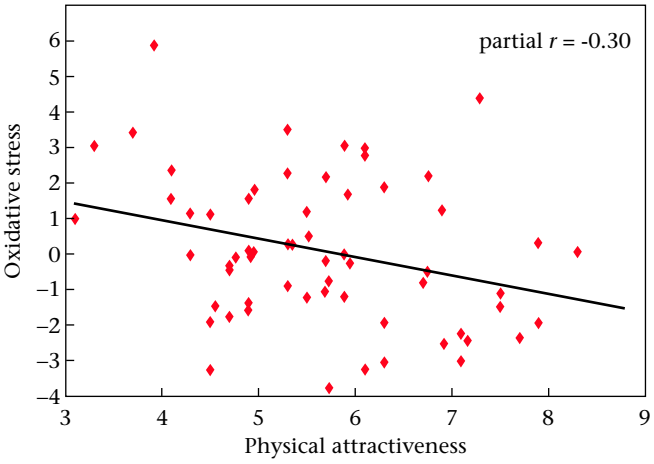

Figure 2. Scatterplot of men's physical attractiveness and oxidative stress. Values are mean-centred residuals, with toxin exposure, smoking, and their interaction partialled out.

Potential Confounds: Dietary Habits and Exercise

With toxin exposure and smoking controlled, our composite measure of healthy dietary habits did not predict composite oxidative stress (partial $r = 0.15$, $N = 70$, $P = 0.229$). Taking of vitamins or herbal supplements also did not predict oxidative stress (partial point-biserial $r = -0.07$, $N = 70$, $P = 0.571$; partial point-biserial $r = 0.00$, $N = 70$, $P = 0.995$; see also Bloomer & Fisher-Wellman 2008). Finally, frequency of physical exercise did not predict oxidative stress (partial $r = -0.04$, $N = 70$, $P = 0.770$). Reports of the extent to which individuals had engaged in physical activity in the last 2 h or the last 2 days similarly did not predict oxidative stress (partial $r = 0.09$, $N = 70$, $P = 0.462$; partial $r = 0.05$, $N = 70$, $P = 0.714$).

Subcomponents of Oxidative Stress

As explained above, we aggregated our measures of oxidative stress to construct a measure that, a priori, should have maximized chances of detecting associations with oxidative stress. We nevertheless asked whether any one component outperformed others. Aggregated across samples, levels of 8-OHdG covaried positively with levels of MDA ($r = 0.26$, $P < 0.05$), consistent with these biomarkers both tapping oxidative stress.

Table 2 lists partial correlations with the two biomarkers and two samples separately. 8-OHdG was significantly associated with FA, attractiveness, health and masculinity, and was marginally significantly associated with birth complications; correlations with MDA were typically weaker, although all correlations were in expected directions.

Samples collected during the laboratory session yielded, on average, slightly higher correlations than morning samples. Our first samples were collected in the laboratory, a time of day after individuals had already engaged in activities. Our second sample was collected in the morning, reflecting oxidative stress during sleep. Given differences in the metabolic demands of these two periods, they may tap quite different aspects of the systems that contribute to oxidative stress. Indeed, circadian variation has previously been documented in oxidative stress biomarkers, and this may be related both to rhythmicity in the formation of ROS due to circadian changes in energy intake and expenditure and exposure to light, and to rhythmic activity of antioxidants, which are upregulated by melatonin (Kanabrocki et al. 2002; Hardeland et al. 2003). Oxidative stress experienced during these different time periods may reflect somewhat different individual differences, which future research should investigate. At the same time, we emphasize that differences between correlations across biomarkers or samples were not statistically robust (all $P > 0.10$). Our data therefore do not permit firm conclusions about one particular biomarker or sampling time possessing stronger relations than any other.

DISCUSSION

A Summary of Findings and Possible Implications

The current study found that men's FA and physical attractiveness predicts their level of oxidative stress. These findings have several potentially important implications.

First, they suggest that individual differences in human oxidative stress are likely to be substantial and important. A standardized measure of variation is the coefficient of variation (CV). We observed large CVs across participants of 33 and 36 for 8-OHdG and MDA, respectively, consistent with past research on healthy samples (e.g. 36 for 8-OHdG in Kimura et al. 2006; Tamura et al. 2006). By contrast, human height has a CV of ~5. Lacy et al. (2000) reported a heritability of approximately 0.2–0.35 in human serum hydrogen peroxide levels, and oxidative stress has substantial genetic components in lizards (Olsson et al. 2008) and kestrels (Costantini & Dell'Omo 2006). If the CV of oxidative stress is 30–35 and h^2 is 0.2–0.3, the CVA (additive genetic coefficient of variation) is 12–19. Important fitness traits tend to have CVAs in this range whereas traits under stabilizing selection tend to have CVAs less than 5 (Houle 1992; Pomiankowski & Møller 1995). Hence, oxidative stress may be an important fitness trait in humans.

Oxidative stress appears to possess substantial temporal stability. Mizoue et al. (2007) reported a correlation of 0.79 between 8-OHdG levels in healthy adults measured 1 year apart. We found that oxidative stress covaries with birth complications in college-aged men, as it does in infants (e.g. Nassi et al. 2009) and children (e.g. Mohn et al. 2007; Chiavaroli et al. 2009), suggesting that some stable individual differences in oxidative stress levels emerge early.

Second, these findings may address the origins of FA. The developmental perturbations that contribute to asymmetrical growth remain largely unknown (e.g. Polak 2003). Possibly, a major cause of asymmetrical growth is oxidative damage to DNA or cellular membranes. Our results indicate that FA is associated with birth complications (see also Livshits et al. 1988), which are themselves linked to oxidative stress in infancy, childhood and adulthood (see references above).

Third, these findings are compatible with the proposal that women evolved to find particular features attractive because they are related to low levels of oxidative stress.

Men that appeared healthy and masculine (e.g. muscular) had low oxidative stress. Possibly, highly fit individuals are attractive and have lower oxidative stress levels. Lifestyle or exercise patterns, however, do not appear to drive the association between attractiveness and oxidative stress.

Our results cannot speak to whether ancestral benefits of a mate's low oxidative stress were direct (e.g. due to greater longevity and health of a mate; see, e.g. Pike et al. 2007) and/or indirect (e.g. heritable factors affecting oxidative stress). Because oxidative stress appears to be an important cause of mutations (e.g. Denver et al. 2009) as well as a function of them, low oxidative stress may offer indirect genetic benefits even if not heritable itself. Hence, physical attractiveness or low FA may be preferred in sires because of indirect benefits associated with low oxidative stress (in testes) and risk of de nova mutation (e.g. Velando et al. 2008), even if weakly heritable (e.g. Cornwell & Perrett 2008).

Alternative Explanations

The above discussion may seem to imply that individual differences in oxidative stress cause differences in developmental instability or physical attractiveness. But other causal scenarios are possible: perhaps individual differences in developmental instability or physical attractiveness cause differences in oxidative stress. Or perhaps each is caused by a third variable.

If individual variation in developmental instability or physical attractiveness causes variation in oxidative stress, there must be some reason why this is so. We examined one plausible idea: that low attractiveness causes psychosocial stress in individuals' lives, which leads to greater levels of cortisol, which may cause increases in oxidative stress. In fact, we found no evidence for this idea. Cortisol levels did not covary with oxidative stress, FA or attractiveness. Partialling out cortisol levels did not reduce the correlations of FA and attractiveness with oxidative stress.

Variations that might cause both attractiveness or developmental instability and oxidative stress include energetic stress or poor diet. Once again, we detected no robust associations of cortisol levels (increased with energetic stress), dietary habits or exercise with oxidative stress. Partialling out these variables from correlations of FA and attractiveness with oxidative stress did not diminish them. These results are inconsistent with these factors being third variables that drive associations with oxidative stress.

More generally, these results suggest that the associations of physical attractiveness and FA with oxidative stress are not due to short-term energetic or psychosocial stresses, as reflected by increases in cortisol levels. Rather, the results are most consistent with associations being driven by physiological processes experienced over a longer period.

Naturally, we cannot rule out the possibility that one of these alternative causal scenarios accounts for the associations we observed, rather than oxidative stress causing developmental instability or attractiveness. Longitudinal research designs that examine how these associations emerge over the developmental life course may be necessary to assess in a more definitive way the causal explanation of these associations.

Unanswered Issues

Even if relatively stable individual differences in oxidative stress cause individual variation in developmental instability and attractiveness, several important issues remain. The first concerns the causes of individual differences in oxidative stress. Oxidative damage occurs when the production of ROS outpaces the production of antioxidants that neutralize them. Variation in oxidative stress, then, can be due to variation in the rate of production of ROS or the rate of production of antioxidants. Above, we noted that some individuals may create particularly high levels of ROS or more harmful ROS, for example, as a function of the rates at which superoxide anion is created or converted into more damaging radicals, themselves functions of mutations, nutrition and other factors (e.g. Murphy 2009). But individuals may alternatively experience high levels of oxidative stress because they have a lower capacity to produce antioxidants, possibly because they have lower overall energy budgets. We suspect that the latter source of variation was less important in our study, simply because our sample of males was generally well fed; indeed, we found no correlations between oxidative stress and weight or quality of diet. But additional research is needed to address this issue. For instance, research could examine whether antioxidant capacity (e.g. rate of production of superoxide dismutase or catalase) is associated with men's FA and attractiveness, and whether variation in production of ROS is critical.

A second outstanding issue to be addressed through further research concerns whether similar associations exist in women.

Broader Implications for Theory and Research across Species

As emphasized in the Introduction, theory concerning the role of oxidative stress in shaping life history evolution and sexual selection processes applies broadly to aerobic organisms. To date, limited empirical research has explored these roles. There is no reason why our findings for a human population should not

generalize to some other species, however. Research questions we asked might be fruitfully addressed in work on other species.

Work on oxidative stress and sexual signals in other species performed to date has largely examined differences in ability to afford signals that require reductions in allocations to immune function or increases in allocations to sexual signals. Individuals may differ in the extent to which they pay costs in currencies of oxidative stress for these reallocations. Those that pay the lowest costs are those that can afford the largest signals. Work by Mougeot et al. (2009) and Alonso-Alvarez et al. (2007), done on zebra finches, supports these ideas.

The current study was based on similar ideas. But in contrast to studies on zebra finches, we did not manipulate parasite load or testosterone level. Rather, we examined the correlation of attractiveness with oxidative stress. The correlation we documented may emerge as a result of differential costs of signals in currencies of oxidative stress. But, our study did not use experimentally manipulations to document these differential costs.

If, within a species, differential costs of signalling in currencies of oxidative stress lead to differences in levels of signalling, individuals that signal most strongly need not experience the lowest levels of oxidative stress, as documented in models by Getty (2002) and Kokko et al. (2002). Whether strong signallers end up healthier (e.g. have lower oxidative stress) or unhealthier (e.g. have greater oxidative stress) depends on particulars of the sexual selection system. In Kokko et al.'s (2002) modelling work, a major factor influencing the direction of the relationship is strength of sexual selection. In most sexual selection systems, the most attractive males (those with greatest mating success) end up having greater viability, too (see Kokko et al. 2003; also Jennions et al. 2001). When sexual selection (and reproductive skew) become extreme, with a few males being big winners and many of them remaining unmated, the most attractive males may signal to such an extreme that they end up less viable (despite paying lower marginal viability costs for signal enhancements). We predicted that attractiveness would be associated with lower levels of oxidative stress based on the assumption that human populations, currently and ancestrally, appear to be typically characterized by modest (though varying) levels of sexual selection (e.g. Brown et al. 2009). In species characterized by extremely strong sexual selection on males, attractive, sexually successful males may in fact possess higher levels of oxidative stress. Comparative work on our research question could inform current models of sexual selection.

Future work on humans should examine more directly whether the associations we observed are due to differential costs to sexual signalling. We found no association between testosterone levels and oxidative stress. Nor did we find associations of attractiveness and FA with testosterone level. If attractive individuals could afford larger levels of testosterone because of lower marginal costs, one may well expect them to have greater testosterone levels. Although we found no association between attractiveness and unstimulated testosterone levels (see also Peters et al. 2008), this does not rule out a role for testosterone. For instance, Pound et al. (2009) found that testosterone responses to competition rather than basal levels of testosterone relate to facial masculinity.

More generally, our findings may importantly contribute to literature on human sexual attraction by, as already noted, suggesting a number of potentially fruitful avenues for future investigation, ones that may yield insights into previously unidentified factors that affect the development of phenotypic features under sexual selection and contribute to the shape of human life histories. They also, however, suggest potentially fruitful avenues of investigation of the role of oxidative stress in the sexual selection systems and life histories of other species.

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