

THESIS

RELATIONSHIP QUALITY AND MEN'S OXIDATIVE STRESS

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ABSTRACT

RELATIONSHIP QUALITY AND MEN'S OXIDATIVE STRESS

The association between oxidative stress and quality of romantic relationships was investigated in a sample of 98 college males. Given a postulated life history trade-off between current and future reproductive potential, men currently in higher quality romantic relationships may expend less general mating effort (i.e., less energy allocation to finding, attracting, and competing for new mates) than single men or men in lesser quality relationships. Reduced mating effort may allow greater allocation of energy to anti-oxidant defense systems, and increased resistance to oxidative damage by reactive oxygen species (ROS). Consistent with this prediction, men who reported being in higher quality romantic relationships (i.e., relationships characterized by greater mutual investment and emotional engagement) had significantly lower levels of oxidative stress than men lacking such relationships. Neither testosterone nor cortisol mediated the effect. Due to the correlational nature of the research design, causal relations are unclear; theoretical interpretations are discussed. Resistance to oxidative damage could be a physiological mechanism by which the experience of being in a higher quality romantic relationship manifests in direct health benefits. Alternatively, men with inherently greater resistance to oxidative damage (due to less ROS production, better functioning anti-oxidant defense, or both) may be more likely to achieve such relationships, owing to pre-existing superior quality or fitness.

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Chapter 1: Introduction

Relationships and Health

Ample evidence indicates that people in high quality romantic relationships experience significant health benefits. Marriage, for instance, has well-documented benefits: married adults experience lower morbidity and mortality than their unmarried counterparts across a variety of acute and chronic health conditions, including cancer, heart attacks, and surgery (Chandra et al., 1983; Goodwin et al., 1987; Gordon & Rosenthal, 1995; House et al., 1988).

Originally, it was thought that simply being in a relationship was the key factor responsible for these benefits, particularly for men; unmarried women have 50% greater mortality than married women, compared with 250% for men (Ross et al., 1990). Further analyses, however, indicate that relationship quality is central to the effects. Unmarried people are happier, on average, than unhappily married people (Glenn & Weaver, 1981), and troubled marriages have negative health consequences. The relationship quality responsible for this variation may be perceived partner responsiveness, generally defined as the belief that relationship partners are cognizant of, sensitive to, and behaviorally supportive of one's needs (Reis et al., 2004). Negative and hostile behaviors during marital conflict discussions are related to elevations in cardiovascular activity, alterations in hormones related to stress, and dysregulation of immune function (Robles & Kiecolt-Glaser, 2003). These physiological changes could have long-term implications for health outcomes, as marital factors appear to play a prominent role in a number of distinct points in the disease trajectory, including possible etiology (Hibbard & Pope, 1993), symptom exacerbation in chronic degenerative illnesses (Vitaliano et al., 1993), and recovery following a life-threatening medical event (Helgeson, 1991).

While the outcomes of positive romantic interactions have been identified, the precise physiological mechanisms by which romantic interactions impact men's and women's health remain largely unknown. Important roles for cortisol and other hormones have been postulated. Elevation of blood pressure, cortisol, norepinephrine, and prolactin (a hormone that counteracts the effects of dopamine, which is responsible for sexual arousal) have been documented in response to negative behavior during relationship conflicts, particularly in women (Kiecolt-Glaser et al.,1996); positive romantic interactions involving warm partner contact (kissing and hugging) appear to increase peripheral circulating serum proteins, which may in turn promote health and feelings of well-being (Matsunaga, 2009). But it's not obvious how health benefits of being in a supportive romantic relationship—such as reduced risk of cancer—arise from reduction of psychosocial stressors or related phenomena. Multiple physiological systems may be involved, and quite possibly, some of the important physiological mediators between quality of relationships and health benefits remain unidentified. Further study of these phenomena, as well as the search for their physiological bases, may benefit from an evolutionary perspective.

Chapter 2: Review of Related Literature

Human Mating Systems

Human mating systems occupy an interesting niche among mammals. Very few mammals exhibit monogamous adult relationships or biparental care of young. Yet, it appears, humans have evolved to form pair-bonds and make commitments between men and women in regards to raising offspring (e.g., Kaplan et al., 2000). Not every adult human will form a pair-bond, and some pair-bonds occur within polygynous or polyandrous arrangements. Nevertheless, in both traditional societies and modern Western ones, most human adults enter some form of socially or sexually monogamous relationship (marriage, cohabitation, extended families) sometime during the lifespan (Hamon & Ingoldsby, 2003). Within pair-bonds, both men and women invest in offspring. Furthermore, in some well-studied traditional foraging societies, many adults will remain in the same pairs throughout most or all of their reproductive lifespans (e.g., Kaplan et al., 2000).

Life History Theory

The evolution of human mating systems can be thought of in terms of life history theory, a conceptual framework within modern evolutionary biology. Organisms are viewed as entities that harvest energy from their environments and allocate it to fitness-enhancing activities. Traditionally, life history theory carves up allocations into two broad domains: survival effort (e.g., somatic repair, immune function) and reproductive effort (e.g., mating, parenting). Because energy is a finite resource, trade-offs are inherent: energy allocated to one life task (e.g., growing to larger size) cannot be simultaneously allocated to another task (e.g., reproducing) (Roff, 1992; Stearns, 1992). An organism's optimal allocation-strategy (favored by natural selection) will be the one that maximizes fitness, for that organism in its specific environment, in the face of such trade-offs.

Mating Effort Versus Parental Effort

Within the domain of reproductive effort, energy allocations can be further delineated into two kinds: mating effort and parental effort (Low, 1978). Mating effort is effort exerted to identify, attract, and compete for mates. Parental effort is effort exerted to increase the quality or quantity of offspring produced. In most mammalian species, males and females specialize in different forms of reproductive effort. In the large majority of these species, females account for virtually all parental effort. As females consist of a limited pool of reproductive resources (e.g., Trivers, 1972), males compete with one another to gain access to and mate with females, thereby tapping into female parental investment for their own offspring. Males, then, account for the large majority of mating effort in these species. In species characterized by biparental care (e.g., most birds and some mammals, including humans), however, both males and females exert parental effort, and females may account for a higher proportion of mating effort in the species. In many species of birds, males iteratively exert large amounts of mating effort and parental effort: To secure a mate early in a breeding season, a male exerts much mating effort; once mated, a male increases parental effort at some expense of mating effort (though, as the current mate must be retained and access to additional, extra-pair mates could increase fitness, some level of mating effort is maintained). The following season, the process begins anew.

Current Versus Future Reproductive Potential

An additional trade-off is implicated between energy allocated to current reproductive possibilities versus energy invested in resources for future potential matings. Consequently, to maximize their reproductive success, males benefit from investing strategically, context determining the most advantageous mating strategy: whether to invest in a current mate, or, to divert energy from current matings with low potential reproductive gain to future, potentially more valuable matings. A male's cost-to-benefit ratio, and ensuing strategy, may be influenced

by factors such as the male's quality and attractiveness to females (as healthier, more attractive males may have greater access to future matings with extra-pair partners), the male's age (since more advanced age entails diminishing returns in future reproductive potential), the availability of female mates, or the quality of the current female mate versus potential alternative mates. In species where females also exert mating effort, female life histories may also be shaped by these factors. Female common gobies, for instance, have been found to increase current reproductive effort (by increasing clutch size) when future access to males is uncertain (Heubel, 2008).

Physiological Mechanisms Responsible for Modulation of Effort

The concept of trade-offs- such as trade-offs between mating effort and parenting effort, or energy allocated to current reproductive potential versus future reproductive potential- is central to our understanding of life history evolution, yet the underlying physiological mechanisms orchestrating these effects have been little studied (Monaghan et al., 2009). One avenue that has received some attention from a life history perspective is the testosterone endocrine system, which appears to modulate mating effort in males. Testosterone can be thought of as a messenger in a distributed communication system through which processes can be upregulated or downregulated in coordinated ways. In a broad conceptual sense, increases in testosterone have been thought to upregulate physiological and psychological processes involved in mating effort (e.g., increases in muscle mass, increases in physiological capacities for competition, greater psychological attunement to male-male competition). It appears that high testosterone interferes with paternal effort by inspiring greater allocation to mating effort instead. Thus, it is adaptively reduced to foster a paternally investing mode. A substantial literature shows that, in many socially monogamous bird species that exhibit biparental care, males' testosterone levels drop when they become mated or have offspring (e.g., Oliviera, 2004). Human males seem to exhibit a similar pattern: men's testosterone levels are lower when

engaged in exclusive romantic relationships (Burnham et al., 2003; Gray et al., 2004) and their testosterone levels may even rise again after a de-coupling event, such as divorce (Mazur & Michalek, 1998).

Costs of Mating Effort

From a life history perspective, energy allocations to mating effort appear to entail "costs". Until recently, the major cost emphasized by behavioral ecologists and other evolution-minded researchers was immune function or cellular repair (that is, somatic maintenance), and, in species with biparental care, reductions in parental effort (see Ellison, 2003; Bribiescass, 2001.) Some of these costs appear to be mediated through the effects of testosterone: Indirectly, through increased allocation of effort to other tasks, increases in testosterone levels take away energy from immune function and, hence, have immunosuppressive consequences (e.g., McDade, 2003; Alonso Alvarez, 2007). By reducing immunocompetence, increases in testosterone levels render an individual more prone to infectious disease and, hence, morbidity and mortality. Possibly, decreased testosterone levels in happily mated men may account for some of their health benefits via effects on immunocompetence. More recently, other costs of testosterone entailed by trade-offs between mating effort and somatic maintenance have been explored. Specifically, increases in testosterone levels and mating effort may lead to increases in *oxidative stress* (Alonso Alvarez, 2007).

Oxidative Stress, Defined

Oxidative stress, not to be confused with the general stress response or psychosocial stress, is a naturally occurring physiological state resulting from the normal metabolic processes by which all living cells produce energy. During this process of cell respiration, organic substances are oxidized and natural byproducts of the oxidation process are created called reactive oxygen species (ROS), which are free radicals that contain the oxygen atom. ROS are

essential and perform a variety of necessary cell functions. For instance, they play an important role in cell signaling and autophagy, assisting with cellular information processing in response to the microenvironment. The chemical reaction by which ROS and other free radicals are created is termed a reduction-oxidation reaction, or "redox" process, referring to the reduction or gain of electrons by molecules involved in the process. Electrons orbit around the nuclei of atoms, and usually the electrons within each orbital are paired with another electron that's spinning in the opposite direction. The paired electrons keep the molecule relatively stable and thereby less reactive. When one or more electrons, especially within the outer orbital, are unpaired with respect to spin, the molecule becomes relatively unstable, and is consequently more reactive with other molecules. A free radical is a molecule with one or more unpaired electrons in its outer orbital.

Free radicals centered around the oxygen atom (ROS) may be highly unstable, and tend to react rapidly with adjacent molecules. This reaction not only changes the adjacent, target molecule, but often passes the unpaired electron along to the target. This generates a second free radical or other ROS, which can then go on to react with a new target, creating a molecular chain reaction by which the reactivity of ROS is amplified. Components of the living cell (cellular membranes, DNA, and RNA) are susceptible to free radical injury and these chain reactions can have substantial effects on the structure and function of living tissues (for a review, see Bulkley, 2002).

Because these oxidation processes have evolved as functional components of an oxygen-dependent metabolism, they represent a normal part of existence as an aerobic organism. As noted above, ROS production is not damaging in every sense, and is related to adaptive physiological processes as well. Both animals and plants appear to intentionally harness ROS for use as molecular messengers to fulfill a wide range of biological processes, including roles in

the identification of and defense against invading pathogens. At high levels, however, ROS are known to exert very damaging effects on surrounding tissues and DNA.

As a consequence, natural selection has driven the evolution of a number of intracellular defense mechanisms to neutralize or control the potentially destructive reactivity of ROS. This includes the production or maintenance of anti-oxidants in the body: molecules that react preferentially with ROS without passing that reactivity along. Some of these are simple molecules like vitamins E and C, while some are enzymes like superoxide dismutase (SOD) and catalase, which function to react with ROS in cells and render them stable (i.e., scavenge them), thereby lessening cellular damage. Just as there is a diversity of ROS, some more unstable than others, there also exists a diversity of anti-oxidant defense mechanisms, including processes of prevention, interception, and repair. Thus, oxidative stress can be conceptualized as a state of physiological imbalance: An organism whose production of ROS exceeds its anti-oxidative capacity is said to be in a state of oxidative stress (for reviews, see Beckman & Ames, 1998; McCord, 2000; Droge, 2002).

Oxidative Stress, Metrically

Oxidative stress has been little studied from a psychological or evolutionary point of view, although an estimate of organism's net oxidative stress level may be readily, even if indirectly, assessed via non-invasive procedures, such as by lab assay of biomarkers that appear in urine. 8-hydroxydeoxyguanosine (8-OHdG) is the most widely used urinary biomarker of oxidative DNA damage and repair processes, (Mayne 2003; Wu et al. 2004; Tamara et al. 2006) and is secreted into urine upon cellular repair of damaged DNA. Malondialdehyde (MDA) is a common biomarker of lipid peroxidation, reflecting oxidative damage to cell membranes and other tissues (e.g., Mayne 2003), and appears in urine as an endproduct of the oxidative degradation of lipids. The sensitivity and specificity of biomarkers of oxidative stress, generally,

have yet to be validated (Dalle-Donne et al., 2006). Though the efficiency of anti-oxidant repair processes is conflated with amount of ROS production or environmental assault, net or systemic oxidative stress may yet be estimated indirectly by these biomarkers, which reflect end results of the interaction of ROS with cellular DNA or lipids.

There exists wide biologic variation in measures of oxidative stress in humans. A large study of healthy Japanese adults found a coefficient of variation ($CV = 100 \times SD/mean$) of 36 for urinary 8-OHdG (Kimura et al., 2006). If, as another study (Mizoue et al., 2006) found, the correlation between urinary 8-OHdG levels at two points separated by one year is .79, the CV of the stable, reliable, inter-individual component of 8-OHdG values is 32. A study of healthy children found a CV of 39 for 8-OHdG levels (Tamura et al., 2006). Age correlated -.66 with these values. Taking into account only the age-independent variation across individuals yields a CV of 29. Similarly, the CV of serum 15-F_{2t}-isoprostane (also called 8-isoprostane, which, like MDA, is another common biomarker that reflects lipid peroxidation,) was estimated to be 36 in a sample of 25 healthy controls measured at 4 different time points, each separated by 3 months (Kato et al., 2006). These authors estimated the intraclass correlation across values at the different time points to be .56. Hence, the stable inter-individual component of 8-isoprostane in healthy adults appears to be about 27. These values reveal substantial variation across individuals. (A variable such as human height has a CV of approximately 5.) Implications of this variation are unknown and represent a compelling area of investigation, given that highly variable biologic variables which are heritable appear on average to be associated with fitness (Houle, 1992). The inter-individual variation in oxidative stress levels in adults also appears to be somewhat stable over time; as noted above, one study found the correlation between 8-OHdG measured at 2 points separated over a year to be .79 (Mizoue et al., 2006).

Factors that are known to affect biomarkers of oxidative stress in normal adults don't seem to explain much of the variation. Some variation in 8-OHdG or 8-isoprostane in healthy adults appears to be due to exposure to contaminants or toxins, such as heavy metals (e.g., iron, chromium, arsenic; Kimura et al., 2006; Tuomainen et al., 2007) or smoking (though results have been inconsistent; see review in Pilger & Rutiger, 2006; see also Lu et al., 2007). Individuals of lean body mass may experience higher levels of oxidative stress (Mizoue et al., 2007) and some dietary factors may have effects on certain components (for a review, see Mayne, 2003). One study found that, among people with high levels of cholesterol, those who possessed the ApoE-4 allele (a risk factor for Alzheimer's disease) had greater levels of 8-isoprostane than those lacking it (Dietrich et al., 2005). Nonetheless, most of the inter-individual variation in biomarkers of oxidative stress remains unexplained by known factors.

Oxidative Stress, General Knowledge

The notion that oxidative stress is responsible for senescence-related loss of function, due to progressive and irreversible accrual of molecular oxidative damage, was posited by the "free radical theory of aging" (Harman 1956). Since then, clear links have been established between oxidative stress and aging, senescence, and the breakdown of tissue and physiological processes that result in diminished capacity for function (Allen & Tresini, 2000; Finkel & Holbrook, 2000; von Zglinicki, 2002). More generally, it has been implicated in a wide variety of diseases as both cause and outcome (e.g., Cooke et al., 2003). Oxidative stress can cause tumor production and cancerous cell growth (e.g., Gopalakrishna & Jaken, 2000). It appears to contribute to the formation of plaques in Alzheimer's disease and hence is a primary factor in the etiology of this disease (e.g., Markesbery, 1997; Nunomuro et al., 2001; Practico et al., 2001), as well as other neurodegenerative diseases (e.g., Parkinson's; e.g., Beal, 1995; Barnham et al., 2004). Oxidative stress appears to play important mediating roles in metabolic, vascular, and renal disease (see,

e.g., Giugliano et al., 1996; Dhalla et al., 2000; Dzau, 2001; Himmelfarb et al., 2002; Dandona et al., 2004).

Oxidative stress may also be linked to senescence via metabolic rates. Consider the "rate-of-living theory of ageing" (Pearl 1928), one of the earliest theories of aging, which posits that lifespan of a species depends upon, and is limited by, metabolic rate, yielding the "live fast, die young" heuristic to estimate lifespan. Though metabolism has since then been largely discounted as a determinant of lifespan and is not thought to adequately explain the relationship between longevity and metabolism across species, oxidation processes (and ensuing damage) may account for some of the exceptions to the rule. Recent research has suggested that some species may be more prone to oxidative stress than others due to variation in the fatty acid composition of cell membranes, and that if this variation across species is taken into account, the rate-of-living theory of aging may find better support (Hulbert et al., 2007). The metabolic trade-off proposed between metabolic rate and lifespan could possibly be mediated by the production and damaging effects of reactive oxygen species (ROS), as faster metabolism should augment ROS production (Dowling & Simmons, 2009). This presumed trade-off (between rate of living and lifespan) is also consistent with a core genetic model of aging, antagonistic pleiotropy (Dowling & Simmons, 2009), which posits that alleles that increase rate of aging and decrease lifespan will accumulate if these same alleles confer high fitness early in life. In sum, there appears to be a variety of ways by which ROS may be involved in trade-offs between lifespan, metabolic rates and fitness (Dowling & Simmons, 2009).

In humans, most health-related research on oxidative stress has looked at its role in overt disease processes. Very little research has examined markers of oxidative stress in healthy populations, where significant biologic variation exists, and levels appear to be moderately stable across time within individuals. Little is known about the implications of this variation, but

managing oxidative stress is likely to be a major determinant of life histories, as virtually all activities generate oxidative stress, because it results from energy expenditure (Monaghan et al., 2009).

Oxidative Stress and Heritability

Although several environmental factors are documented to influence redox metabolism, relatively little is known about genetic effects. Previous research supports the existence of a mild to moderate genetic heritability of the redox state. Total antioxidant status, as an indicator of redox homeostasis, appears to be heritable (Wang, 2001), although genes regulating redox homeostasis have yet to be identified. Prevalence of free radicals also appears to have heritable components. Consider hydrogen peroxide, one of the oxygen free radicals which is posited to mediate oxidative stress: Heritability estimates from familial correlations in humans revealed that approximately 20% to 35% of the observed variance in hydrogen peroxide production could be attributed to genetic factors (Lacy et al, 2000). Further, a study in a lizard population found that ROS levels showed high heritability and variability among families (Olsson 2008). If ROS levels are heritable in humans, ancestral benefits of a mate's low oxidative stress could be not only direct (e.g., due to greater longevity and health of a mate; see, e.g., Pike *et al.* 2007), but also indirect (e.g., heritable factors affecting oxidative stress). Oxidative stress is an important *cause* of mutations (e.g., Denver *et al.* 2009) as well as a function of them. ROS may have direct effects on genes through mutations in the germ line, and it has been suggested that oxidative DNA damage may produce gene mutations and structural alterations of the DNA, resulting in a heritable mutation (Klaunig et al., 1998). Hence, low oxidative stress may correspond with indirect genetic benefits *even if* not heritable itself.

Oxidative Stress from a Life History Standpoint

From a life history standpoint, anti-oxidation processes function as somatic maintenance effort. That is, it is a cost that organisms pay to maintain their soma and prevent damage to it caused by oxidation. How much effort should an organism pay for anti-oxidation? That clearly depends. The more prone organisms within a species are to extrinsic mortality risks (e.g., predation), the less worth it is to use energy for somatic maintenance and anti-oxidant strategies at the cost of reproduction. And within a species, at particular times of life it may pay to engage in more or less somatic maintenance, depending on the value of other potential allocations of energy. Just as it shapes life history strategies in general, selection should be expected to shape a life history strategy for allocating effort to anti-oxidant strategies. Selection will never produce anti-oxidant strategies that are perfect, however (a prediction following from life history theory's marginal gains theorem), and hence oxidative stress is a natural phenomenon that all organisms experience.

Oxidative Stress and Mating Effort

From a life history perspective, increased mating effort (or parenting effort) may increase oxidative stress in at least two different ways. First, increases in allocation of energy to physiological capacity for physical performance or muscular growth and maintenance (for males) or reproduction (for females) may take away energy from anti-oxidant strategies. Second, these same increases may increase overall metabolic rate (e.g., increased energy production in muscles), which in turn increases production of reactive oxygen species (ROS). Conversely, reduced mating effort may lead to reduced susceptibility to oxidative stress, as a result of either more available energy to allocate to anti-oxidant strategies, or less ROS production. When men find mates with whom they have satisfying relationships, they may reduce mating effort; this reduction in mating effort may lead to decreases in oxidative stress.

Studies in the animal literature have found a pattern consistent with this general idea, suggesting that ROS could indeed be an important player in determining the cost of reproduction, one of the most fundamental life history trade-offs. Studies on the effects of oxidative stress on the breeding parameters of zebra finches are a case in point: When the brood size of captive birds was manipulated, male finches who were co-raising the largest broods experienced a large decrease in their resistance to oxidative stress. A greater number of breeding events was also correlated with less resistance to oxidative damage. An additional study showed that greater clutch size correlated with greater oxidative stress experienced by both parents (Alonso-Alvarez et al. 2004, 2006, Bertrand et al. 2006). Results consistent with this pattern have also been observed in studies with *Drosophila*: In females, stimulated egg production seemed to lower the females' ability to withstand free radical attack (Wang et al., 2001).

Predictions Regarding Oxidative Stress and Relationship Quality

The present hypothesis was theoretically based in an evolutionary, life history framework, presuming a life-history trade-off between mating effort and longevity, as discussed above. It was hypothesized that men who are engaged in a fulfilling, stable pair-bond may be investing their energy in keeping a current mate and providing direct benefits to her that may potentially result in an offspring, as opposed to channeling effort towards attracting and competing to obtain a new mate. Men oriented towards keeping a current mate may exert less energy toward mating effort, as searching for new mates may entail greater energy allocations towards testosterone-enhanced traits. As a result, they might have more effort to invest in other tasks (such as somatic maintenance), experience less oxidative stress, and perhaps, ultimately, lesser rates of senescence. Conversely, men who are in relationships of lesser quality, and men who are single, may be less invested in a current mate and more oriented towards finding a new one. As such, they may expend higher levels of mating effort, pay a greater somatic energy cost,

and experience higher oxidative stress. Therefore, two primary predictions were made: Firstly, it was predicted that men in higher quality romantic relationships (i.e., those that are more emotionally engaging and marked by greater mutual investment) would have lower levels of oxidative stress than men in lesser quality romantic relationships. Secondly, it was predicted that men currently involved in romantic relationships would have lower oxidative stress than single men.

Predictions Regarding Testosterone

Because men's mating effort is thought to be upregulated (in part) by testosterone (as discussed earlier), it was hypothesized that testosterone might mediate the relationship between relationship quality and oxidative stress. Men in higher quality relationships may be expending less mating effort in general, and may have less utility in maintaining higher testosterone levels for the purposes of mating effort (e.g., to maintain sexual display traits, or facilitate sexually competitive behaviors). This may leave more energy to be allocated to somatic effort in the form of anti-oxidant defense, resulting in lower oxidative stress. Therefore, it was predicted that men in higher quality relationships would have lower oxidative stress as well as lower testosterone levels (*low oxidative stress, low testosterone*). Conversely, energy allocations to maintaining high testosterone may entail a fitness cost, and men in lesser quality relationships (who are presumably expending more mating effort) may maintain higher testosterone, albeit while paying the cost of having less energy available to allocate to anti-oxidant defense, and thus higher oxidative stress (*high oxidative stress, high testosterone*).

Other plausible scenarios are conceivable, however. The tendency to experience oxidative stress may be congruous with a generally less efficient system of energy production, which could covary with lower general quality or fitness. Males who are generally lower quality may also produce less testosterone as part of a life history strategy favoring mating strategies

other than intra-sexual competition with other (more fit) males (*high oxidative stress, low testosterone*). And conversely, if higher-quality individuals can "afford" to maintain higher levels of testosterone because they have greater general fitness to begin with, that same greater general fitness may associate with greater resistance to oxidative stress (*low oxidative stress, high testosterone*).

Alternatively, oxidative stress itself could compromise men's ability to invest in costly traits produced by testosterone, and have some direct effect on men's ability to secrete testosterone (*high oxidative stress, low testosterone*). Given the plausibility of these various outcomes, one might expect involvement of testosterone to not be limited to a simple mediator role (e.g., higher relationship quality → reductions in testosterone → reductions in oxidative stress).

Predictions Regarding Cortisol

How cortisol might function as a mediator of an association between oxidative stress and relationship quality is less clear. Cortisol, the primary glucocorticoid stress hormone in humans, helps regulate metabolism of proteins, fats and carbohydrates. Cortisol secretion varies among individuals, and people may be predisposed to react differently to stress. Seckl (2001) proposed that cortisol in utero simultaneously affects programming of the hypothalamic-pituitary-adrenal (HPA) axis affecting cortisol production and physiology affecting metabolic processes and syndromes. When cortisol is secreted in adults, it facilitates a breakdown of muscle protein, leading to release of amino acids into the bloodstream, which are then used by the liver to synthesize glucose for energy. Given that cortisol is thus linked to energy production and mobilization, cortisol levels may well covary positively with oxidative stress, as greater energy conversion could also result in the creation of more ROS as a byproduct, and more oxidative damage.

A positive association between cortisol and oxidative stress could occur by other means. Because cortisol production also increases in response to physiological stress to prepare the body for action and protect it from damage, oxidative stress could possibly be detected by the neuro-endocrine system and trigger the cortisol response. Speculatively, then, higher cortisol might associate with higher oxidative stress levels to the extent that oxidative damage triggers a cortisol response. But by the same process, higher cortisol could also associate with lower oxidative stress levels, to the extent that a triggered cortisol response successfully functions to help the body cope with the threatening event (in this case, oxidative damage), perhaps by mobilizing anti-oxidant defense systems and thereby reducing oxidative stress.

A cortisol response need not be triggered only by physiological stress; psychological stress, too, may cause greater cortisol secretions. Cortisol is particularly sensitive to uncontrollable and social-evaluative psychosocial stressors in humans (e.g., see Dickerson & Kemeny, 2004), possibly including those experienced during negative romantic interactions. It may be mitigated by positive romantic interactions, as factors such as "affectionate communication" received from spouses have been found to predict stress hormone levels in healthy adults (Floyd, 2008). Some researchers have suggested that psychosocial stress contributes to oxidative stress as well (Miyashita et al., 2006; Simon et al., 2006; McGinnis, 2007). If being in a high quality romantic relationship lessens general psychosocial stress levels, this might in turn relate to lower cortisol secretions and lower ROS production. Hence, it was predicted that relationship quality would covary negatively with oxidative stress levels.

Additional Predictions, Regarding Morbidity, Schizotypy, FA and Attractiveness

Four variables were identified that may affect either or both of the two variables involved in the primary hypotheses, possibly by relating to hypothetical confounding factors of the relationship between oxidative stress and relationship quality. These variables are:

1. *Morbidity*: Some research suggests that people with disease may not be as good at balancing intimacy and conflict in their relationships with romantic partners (Seiffge-Krenke, 1997). Proneness to disease or general morbidity, then, could be negatively associated with relationship quality if it predisposes men towards lower quality relationships. It could also be positively correlated with biomarkers of oxidative stress, if morbidity represents generally inferior quality or condition.

2. *Schizotypy*: Men who score higher on schizotypy measures may have behavioral propensities towards having difficulty in attaining or maintaining high quality relationships, as personality factors that constitute higher schizotypy seem to strongly predispose low relationship quality. Therefore, relationship quality may be negatively associated with schizotypy. If schizotypy and oxidative stress are related to general quality or fitness, they may be positively correlated as well.

3. *Fluctuating Asymmetry (FA)*: The primary measure of developmental instability (DI), the imprecise expression of developmental design owing to perturbations of development, is FA, asymmetries in bilateral features symmetrical at the population level thought to be due 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, as ROS-induced damage to DNA or cell membranes may disrupt cell replication; oxidative stress may also coincide with FA if both measures tap general quality or condition. Individual propensities towards susceptibility to oxidative stress, then, may be positively associated with FA. Further, men of inferior quality or condition may be less likely to achieve

high quality romantic relationships, which could generate a negative correlation between FA and relationship quality.

4. *Attractiveness*: Physical attractiveness (as well as low FA) may be preferred in sires due to 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). Hence, high attractiveness may associate with lower oxidative stress as well as higher relationship quality.

Because each of these four variables may represent a predisposing factor that could affect relationship quality, oxidative stress, or both, they were measured and assessed for their potential roles as confounding variables.

Chapter 3: Methodology

General Procedure

Data was collected in a sample of 98 college-age men. During one experimental session, study participants filled out a series of questionnaires, provided a urine sample to assess oxidative stress level, and a saliva sample to assess testosterone and cortisol levels. Participants provided a second urine and saliva sample one week following the initial experimental session.

Primary measures are discussed below.

Measure of Relationship Quality

Relationship quality was assessed with the short form of Ellis's (1998) Partner-Specific Investment Inventory, or PSI scale (Ellis, 1998, see appendix, pg. 47-49). The PSI scale is a multidimensional measure that was developed by Bruce Ellis in 1998, advancing an evolution-based model of the function and content of investment in romantic relationships. Scores on the PSI scale correlate positively with partner's feelings of love and felt security in the relationship, correlate negatively with degree of sexualizing of others by partner, and do not correlate with the self's performance of mate retention behaviors (Ellis, 1998).

The PSI scale administered to participants consisted of two sets of identical items. The first set (hereby referred to as "S-INV", for "self-investment") assessed self-reported investment of the participant themselves in their romantic relationship. The second set ("P-INV", for "partner investment") assessed the perceived investment of their romantic partner. Participants were asked to rate how often they (on the S-INV component) or their partner (on the P-INV component) have performed a variety of behaviors during the last six months (or less, if their relationship has not lasted that long), rating items from 0 ("never") to 4 ("very often"). Hence, in regression analyses, the first and second sets of items may be considered separately as predictors, or they can be summed and averaged to assess overall, reciprocal investment in the relationship

("INV", for overall investment). As an aggregate of both subscales (S-INV plus P-INV), "INV" is assumed to reflect the construct of "relationship quality" with the best accuracy.

Items on the scale were endorsed on a 5 point scale, and included statements such as, "I make and discuss plans for our future", "I share my feelings with my partner", and "I want to have sex with my partner." Additionally, participants answered a question about relationship status and length of relationship (see appendix, pg. 49).

Measure of Oxidative Stress

Based on previous literature, two biomarkers of oxidative stress were selected, both of which were assayed in urine: 8-hydroxydeoxyguanosine (8-OHdG), a biomarker of oxidative damage to DNA, and malondialdehyde (MDA), a biomarker of lipid peroxidation (Mayne, 2003).

8-OHdG (8-hydroxydeoxyguanosine) is the most widely used measure of oxidative damage to, and repair of, DNA. Although oxidation of DNA can lead to many products, oxidation of the C-8 of guanine is one of the most common oxidative events, and results in a mutagenic lesion that produces predominantly G-to-T transversion mutations (Mayne, 2003). 8-OHdG has been widely and successfully used to measure and investigate oxidative stress associated with a variety of health conditions (see, e.g., Cooke et al., 2003). We used ELISA kits manufactured by the Japan Institute for the Control of Aging for assays. Assays were performed in duplicate (see Tamura et al., 2006).

Lipids are a major target for attack by free radicals, which results in lipid peroxidation. Lipid peroxidation of cell membranes can alter their biophysical properties and inactivate membrane-bound receptors and enzymes, thereby disrupting normal cellular function. Lipid peroxidation can also produce highly reactive aldehydes, which may amplify cellular damage. Malondialdehyde (MDA) is a widely used measure of peroxidation (e.g., Mayne, 2003). We

used ELISA kits distributed by Northwest Life Sciences Specialties for assays. Assays were performed in duplicate.

Both 8-OHdG and MDA were standardized against creatinine measured in urine, to control for levels of hydration and total energetic utilization in the period in which levels of these measures have accumulated in urine. Creatinine levels were quantified colorimetrically using the Jaffee reaction (Tausky, 1954). All creatinine assays were performed in quadruplicate.

To both increase reliability of measurement and to assess reliability, two samples of urine were obtained per individual, one week apart. During the experimental 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. Upon delivery, we froze it at -20°C . Urinary assays were conducted in the Hominoid Reproductive Ecology Laboratory at UNM under supervision of Dr. Melissa Emery Thompson.

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 predictions, 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 ($N = 71$). We found no associations (linear or curvilinear) between biomarker levels and time since waking and, hence, did not adjust for time of collection.

Measures of Hormones

Two samples of saliva were obtained per individual. Saliva was collected once during the lab session and once the following week. A drop of sodium azide was placed in each test

tube to inhibit bacterial growth and stabilize cortisol and testosterone levels. The time at which participants awoke and the date and time of the collection was recorded. Saliva samples were assayed for testosterone and cortisol using radioimmunoassay techniques. Samples were standardized against creatinine and were assayed in duplicate. First saliva samples (taken during the lab session) were collected at various times of day; second saliva samples were all collected in the morning upon waking. The linear and quadratic components of time since waking were partialled out to adjust for daily fluctuations of testosterone and cortisol levels. Salivary assays were conducted in the reproductive ecology laboratory at UNM under supervision of Dr. Melissa Emery Thompson.

Additional Measures: morbidity, schizotypy, FA, and attractiveness

Morbidity was assessed via questionnaire, by the sub-factor of "susceptibility to disease" on the Perceived Vulnerability to Disease (PVD) scale (see appendix, pg. 50) which correlates with how often people get sick (Duncan et al., 2009). Participants responded to scale items using a 7-point likert scale, rating each from 1 ("strongly agree") to 7 ("strongly disagree"). The PVD scale has demonstrated sufficient internal reliability and adequate construct validity in the past, and correlates with how often people get sick (see Faulkner et al., 2004 for a review).

Schizotypy was assessed via questionnaire, by three scales combined into one schizotypy assessment (77 items total, see appendix, pg.51-53): the Schizoid Taxon Scale (STS, 7 items), the Magical Ideation Scale (MIS, 30 items), and the Social Anhedonia Scale (SAS, 40 items). The Schizoid Taxon Scale (STS, Golden and Meehl, 1979), is a measure of schizotypic symptoms, including cognitive slippage, social anhedonia, and ambivalence. The Magical Ideation Scale (MIS, Eckblad & Chapman, 1983) is a measure of "a belief...of the possibility that events which, according to the causal concepts of this culture, cannot have a causal relation with each other" (p. 217). The Social Anhedonia Scale (SAS, Eckblad et al., 1982) is a measure of

pleasure derived from interpersonal relationships and includes items that identify a lack of interest in social relationships. Participants indicated if items were "mostly true" or "mostly false". The schizotypy composite variable used in analyses was created through a principal components analysis (PCA) on the three scales. The first principal component accounted for 47% of the variance in the three variables (eigenvalue = 1.42). Loadings on the component were .83, .40, and .76 for the MIS, SAS, and STS measures, respectively.

Fluctuating asymmetry (FA) was assessed by measuring 10 bilateral features: ear width, ear height, wrist width, elbow width, lengths of four fingers, ankle breadth, 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 et al. 1998, 2005; Thornhill et al. 1999, 2003). Aggregate FA measures made by two different measurers applying these procedures correlate .7 (Gangestad et al. 2001). We asked each individual whether he had broken, sprained, or injured any feature measured and, if so, substituted the mean asymmetry if asymmetry exceeded it. This procedure, which affected 3.5% of all asymmetry values, eliminates large asymmetries caused by injury.

Because a single trait's FA taps organism-wide developmental instability very weakly (e.g., Gangestad & Thornhill 1999; Van Dongen 1998; Whitlock 1998), we aggregated the FA of all 10 features into a composite index. To create the composite measure, we (a) averaged the two right-side and the two left-side measures for each trait for each individual, (b) took the absolute difference between sides for each trait for each individual, (c) standardized each trait's FA by dividing these absolute differences by the mean trait size for the sample, and (d) 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 .46, the range .72 to 2.88. These values are similar to previous studies (e.g., Furlow et al. 1997; Gangestad et al. 1998, 2005; Thornhill et al. 1999, 2003).

Reliability of measurement was examined for each trait's FA. The mean correlation between unsigned, absolute differences was 0.77 (range 0.72 to 0.89, all $P < 0.00001$) and between signed differences 0.88 (range 0.84 to 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).

Attractiveness was assessed accordingly: Each participant stood against a blank wall. With a digital camera, we took two photos: one of his full body (clothed), and one of his face alone. Participants were asked to look straight ahead, with a neutral expression. We requested written consent to have photos 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, each rater viewed both photographs of a man when making her assessment. Each woman also rated the healthiness and masculinity of each man's appearance (composite $\alpha = 0.82$ and 0.79, respectively).

We also asked participants whether they smoked or had recently been (or were regularly) exposed to environmental toxins (see appendix, pg. 54), so that environmental variables likely to impact oxidative stress level could be controlled in our analyses.

Analysis Techniques

To test the primary prediction, that oxidative stress is positively associated with relationship quality, univariate general linear model analyses were conducted. First, the

association of the composite oxidative stress scores with an aggregate measure of relationship investment (INV) was examined, followed up by analyses with the two sub-components of the PSI scale (self-investment in the relationship, S-INV, and perceived partner investment, P-INV). These associations were evaluated in multiple regression analyses. In each, INV, S-INV, or P-INV was entered as a predictor of oxidative stress, controlling for toxin exposure and cigarette smoking. The sample size of participants for whom full oxidative stress information was obtained who were also in relationships (i.e., who filled out the PSI scale) was 29. Within this sample of 29 participants, the hypothesis tested was that biomarkers of oxidative stress would be positively associated with measures of relationship investment: specifically, that lower oxidative stress would correlate with greater relationship investment.

To test the secondary prediction, that oxidative stress is lower in men in relationships than single men, a univariate general linear model analysis was conducted, examining the association of the composite oxidative stress scores with relationship status. Relationship status (assessed via one item, see appendix, pg. 49) was entered as a categorical predictor and the oxidative stress composite was entered as the dependent variable.

To test predictions about testosterone and cortisol as potential mediating variables, as well as to test potential effects of morbidity, schizotypy, FA, and attractiveness, I examined the partial correlations of each variable with relationship quality, and with oxidative stress, once again controlling for toxin exposure and cigarette smoking.

Directed tests were used to evaluate the hypotheses for all predicted associations. A directed test allocates .04 of the total .05 Type 1 error rate to the predicted direction, and .01 to the unpredicted direction (e.g., Rice & Gaines, 1994).

Chapter 4: Results

Controlled Variables

Previous research has found oxidative stress to be significantly affected by certain environmental variables, particularly exposure to toxins such as heavy metals and cigarette smoke (Luo et al., 2009; Carnevali et al., 2003). When exposure to toxins and cigarette smoking were entered as predictors of oxidative stress in a multiple regression model, these effects were found in the present sample as well. Self-reported exposure to toxins (such as in the workplace, e.g., inhalation of fumes or car exhaust) predicted oxidative stress, $F(1, 69) = 5.09, p < .02$, and current use of cigarettes predicted oxidative stress, $F(1, 69) = 2.66, p = .067$. Thus, the two quantitative variables of both toxin exposure and current smoking were entered as controls in all of the analyses to follow.

Primary Analyses: Oxidative stress and relationship quality, amongst men in relationships

A regression analysis was conducted, with the composite score of oxidative stress entered as the dependent variable and INV entered as a quantitative predictor. INV was the composite of self-reported investment (S-INV) and perceived partner investment (P-INV), and as such, was theoretically our best operationalization of the construct of "relationship quality". Within the subset of men in relationships ($N=29$), we detected a robust negative association between the overall aggregate composite of oxidative stress and INV, $F(1, 25) = 5.05, p < .01$ (see *Figure 1, below*).

This analysis was followed up with separate analyses of the two subcomponents of the primary measure, self-investment and partner-investment, to see if one was driving the effect more strongly. S-INV also predicted oxidative stress, $F(1, 25) = 5.33, p < .01$, as did P-INV, though not significantly, $F(1, 25) = 1.73, p = .08$. The plot below (*Figure 1*) shows how relationship quality associated with oxidative stress (using unstandardized residuals for each

variable). Each dot represents one of the 29 men who completed the PSI scale (i.e., who was in a relationship, and for whom we had a measure of relationship quality).

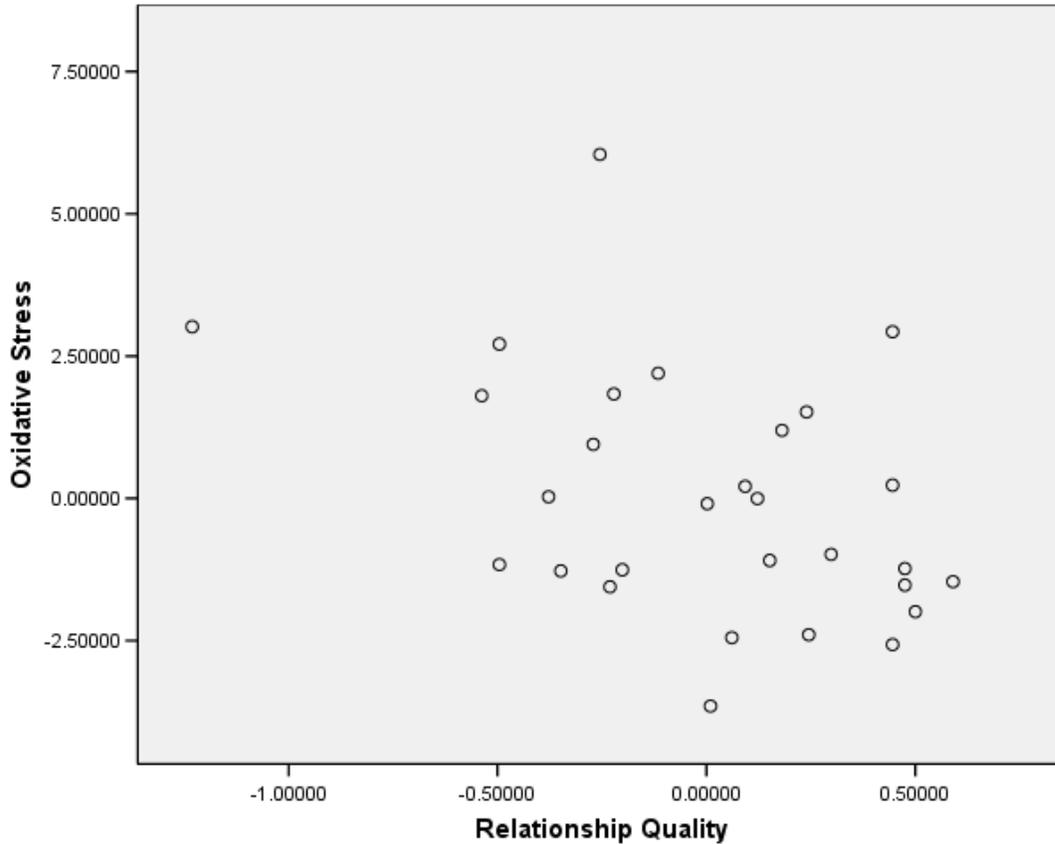


Figure 1. Relationship quality plotted against oxidative stress.

Of the 29 individuals represented in the plot, 8 reported either current smoking or exposure to toxins. 5 of these 8 men had oxidative stress levels below the 0.00 mark, however, as noted, the relationship between oxidative stress and relationship quality remains when controlling for these variables.

Secondary Analyses: Oxidative stress in men in relationships versus men not in relationships

An additional regression analysis was conducted, replacing "relationship status" for relationship quality, as a categorical predictor. We did not detect a significant difference in oxidative stress between men who reported being in relationships versus men without romantic

partners, $F(1, 69) = .29, p = .368$. Interestingly, there generally appeared to be increasing mean oxidative stress levels as men moved on a continuum from being in a relationship (mean oxidative stress level = $-.0149$), to not being in a relationship ($.0667$), to dating multiple people ($.1855$), see *Figure 2*, below:

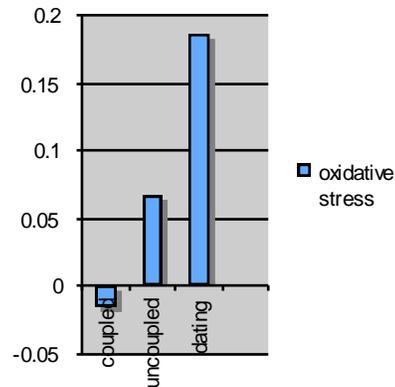


Figure 2. Oxidative stress variation across relationship status.

Though these mean differences were not significantly or reliably different, speculatively, they could suggest that men dating multiple people are expending more mating effort, as we've conceived of it, than either men in invested relationships or single men. Future research could examine whether these differences are robust.

Analyses of Possible Mediating Variables: Testosterone, cortisol

Testosterone and cortisol were assessed as possible mediators of the associations between oxidative stress and relationship quality. Though they were significantly correlated with each other, $r(96) = .29, p < .004$, 2-tailed, or, amongst men in relationships only, $r(39) = .31, p < .05$, 2-tailed, neither was correlated with oxidative stress in this sample. Within the subsample of men in relationships, oxidative stress and testosterone (averaged across two samples) had virtually no linear association, $r(25) = -.03, p = .555$; Oxidative stress and cortisol were non-significantly associated in the predicted direction, $r(25) = .12, p = .335$.

Neither testosterone nor cortisol was associated with relationship quality. INV did not significantly covary with testosterone, $r(37) = .06, p = .450$, or cortisol, $r(37) = .06, p = .455$. Further, when either testosterone or cortisol was entered into a regression analysis as a controlled factor, the relationship between oxidative stress and relationship quality remained unchanged. Were either factor a mediator, this relationship should have been attenuated.

Because testosterone levels are known to fluctuate diurnally, and because the two saliva samples were collected at different times of day, we examined each sample separately as a predictor of oxidative stress. The first testosterone sample (collected during the daytime) was a better predictor of oxidative stress than the second sample (collected in the morning upon waking). However, even using only these first sample values as a predictor, the association with oxidative stress was still non-significant amongst men in relationships, $r(25) = -.13, p = .319$. Further, in our larger sample of men for whom we had complete oxidative stress data, regardless of relationship status, there was no correlation between either testosterone or cortisol and oxidative stress, $r(66) = .02, p = .528$ for testosterone; $r(66) = .10, p = .273$ for cortisol.

In summary, these analyses provide no support for either testosterone or cortisol as a mediator of the relationship between oxidative stress and relationship quality.

Analyses of Possible Confounding Variables: morbidity, schizotypy, FA, attractiveness:

The primary finding that oxidative stress and relationship quality are related raises the issue: does relationship quality directly affect oxidative stress, or does a third variable affect both and generate the association between the two? The primary association could arise spuriously due to an unknown confounding variable. As noted previously, four variables were assessed for their potential impact as confounding variables: morbidity, schizotypy, FA, and attractiveness. If any of these factors indeed tap an underlying trait (such as overall superior genetic quality) that led to the primary association found, they should be correlated with both

oxidative stress and relationship quality. Further, they should be correlated with oxidative stress, not only amongst men in relationships, but in the sample of unmated men as well.

Morbidity

Morbidity (as estimated by the subscale of susceptibility to disease on the PVD scale) was uncorrelated with INV, $r(37) = .04, p = .516$, uncorrelated with oxidative stress amongst men in relationships, $r(25) = -.02, p = .566$, and uncorrelated with oxidative stress in the sample at large, $r(66) = -.02, p = .566$.

Schizotypy

Schizotaxia and magical ideation were considered independently as predictors, but as they correlate with each other, $r(93) = .37, p < .000$, and purportedly both tap the concept of peculiar thinking, they were also combined into a composite measure referred to here as the "schizotypy composite".

Schizotaxia and magical ideation were both correlated with INV, schizotaxia, $r(37) = -.38, p < .01$; magical ideation, $r(37) = -.57, p < .000$. The schizotypy composite correlated with relationship quality, $r(37) = -.53, p < .000$.

Within the sample of men in relationships for whom we also had complete oxidative stress data, (N=25), schizotaxia and magical ideation were positively associated with oxidative stress, though associations were only marginally significant: Oxidative stress was positively associated with magical ideation, $r(25) = .35, p < .05$, positively (though non-significantly) with schizotaxia, $r(25) = .26, p = .123$, and positively with the schizotypy composite, $r(25) = .34, p < .05$.

However, the relationship between schizotypy and oxidative stress was not robust across our sample at large. Including men both in and not in relationships, the relationship between the schizotypy composite and oxidative stress was reduced to $r(66) = .09, p = .295$. Associations

with sub-components, schizotaxia and magical ideation, were non-significant in the larger sample as well, $r(66) = .05, p = .427$, and $r(66) = .09, p = .288$, respectively.

Further, amongst men not in relationships, magical ideation was not significantly correlated with oxidative stress, $r(37) = .04, p = .821$, and the association with schizotaxia, non-significant as well, also reversed direction, $r(37) = -.20, p = .223$.

This pattern of associations may suggest that relationship status interacts with schizotypy to predict oxidative stress. A univariate general linear model analysis testing for this interaction effect yielded a non-significant finding, $F(2,69) = 1.44, p = .168$, providing no credible evidence of an interaction effect. Nonetheless, it could be that schizotypy predisposes low relationship quality, and low relationship quality leads to oxidative stress, thus creating an association between schizotypy and oxidative stress, but only in men in relationships. This possibility could be assessed more rigorously in future research.

FA

Amongst men in relationships, FA was not significantly correlated with INV, $r(37) = -.23, p = .104$. But FA was significantly correlated with oxidative stress $r(25) = .41, p < .02$. FA significantly correlated with oxidative stress in the sample at large, as well, $r(66) = .22, p < .04$.

When FA is controlled, the association between oxidative stress and relationship quality (INV) still exists, weakened, yet marginally significant, $r(24) = -.32, p = .066$. Associations between oxidative stress and scale sub-components were weakened as well (S-INV, $r(24) = -.30, p = .089$; P-INV, $r(24) = -.23, p = .165$).

Attractiveness

Physical attractiveness and INV were uncorrelated, $r(35) = .08, p = .399$. A significant association was found, however, between attractiveness and oxidative stress. Attractiveness

negatively predicted oxidative stress, in mated men, $r(23) = -.33, p = .064$, and in the sample at large, $r(59) = -.25, p < .03$. When physical attractiveness is controlled, the relationship between relationship quality and oxidative stress is weakened, but still marginally significant, $r(22) = -.309, p = .088$.

Analysis of Oxidative Stress Measure

Aggregated across samples, levels of 8-OHdG covaried positively with levels of MDA, $r(92) = .28, p < .004$, consistent with the idea that both biomarkers are tapping oxidative stress. For neither biomarker, however, did concentrations in first and second samples significantly covary, $r(66) = .08, p = .326$ for 8-OHdG and $r(66) = -.06, p = .391$ for MDA, respectively.

As noted earlier, each biomarker purportedly reflects different kinds of oxidative damage, 8-OHdG reflecting DNA damage, and MDA reflecting damage to lipids, such as cellular membranes. Separate analyses on the two biomarkers suggest that associations between oxidative stress and relationship quality are more strongly driven by 8-OHdG than MDA. Analysis of the correlation of each biomarker separately with relationship quality, averaging across both sample times, reveals that 8-OHdG correlated significantly with INV, $r(25) = -.41, p < .02$, while the correlation between MDA and INV was non-significant, $r(25) = -.21, p = .182$. However, the difference between these two correlations is non-significant ($p > .10$). Hence, the difference observed could simply be due to sampling variability.

Biomarker measures were also compared individually across sampling times. Samples collected during the lab session yielded, on average, slightly higher correlations than morning samples: The correlation between 8-OHdG and INV is strengthened by considering only the lab sample (daytime) instead of the average of the two samples (lab sample, $r(25) = -.47, p < .008$; morning sample, $r(25) = -.12, p = .34$, composite of both samples, $r(25) = -.41, p < .02$. For MDA, the lab sample was also a better predictor of INV (though less pronouncedly so, $r(25) = -$

.22, $p = .106$, versus morning sample, $r(25) = .02$, $p = .581$). Averaging across both biomarkers, lab samples were better predictors of INV: (lab sample, $r(25) = -.40$, $p < .02$; morning sample, $r(25) = -.09$, $p = .405$). However, again, differences between correlations across samples were not statistically robust ($p > .10$). The data therefore do not permit firm conclusions about one particular biomarker or sampling time possessing stronger relations than any other.

In light of other studies that have demonstrated strong temporal stability across a year ($r = .79$ for 8-OHdG; Mizoue et al. 2006), it is somewhat surprising that our study yielded little stability of oxidative stress in two samples. At this point, it is unclear why stability was low. One possible reason is that we sampled two different conditions. Our first samples were collected in the lab, a time of day after individuals had already engaged in activities. Our second sample was collected first of the morning, reflecting oxidative stress during sleep. Given differences in the metabolic demands of these two periods, they may tap different aspects of the systems that contribute to oxidative stress. Oxidative stress experienced during these different time periods may reflect somewhat different individual differences, which future research should investigate. Alternatively, we cannot rule out the possibility that sampling variability (i.e., sample-specific fluctuations in correlations across any two studies) led to low stability in this sample.

It appears that the two biomarkers we chose to estimate oxidative stress (MDA and 8-OHdG) were both sound measures, but MDA assay results were somewhat more ambiguous to interpret than 8-OHdG results. The lowest sensitivity of the assay was 0.625 for MDA, 0.5 for 8-OHdG, which is common practice. For MDA, variation within subjects (for two samples from the same individual) was 46%, and variation across subjects was 46%. For 8-OHdG, variation within subjects was 30%, and across subjects was 59%, making 8-OHdG seem like a more promising measure.

We calculated inter-assay coefficient of variations (CVs), to determine if differences in sample determinations could be due to different assay conditions, and intra-assay CVs, to determine reliability of measurement within assays. For MDA, the average inter-assay CV was 16.9%. The CVs of the two controls which represent the range of most samples (mean +/- 1 standard deviation, standards 3 and 4) were 10.9% and 30.8%. Given that the acceptable range of inter-assay CVs is generally considered to be 10-18%, 30% may seem high, however, it may well be realistic for this particular compound. The higher inter-assay CV value may also reflect that our sample consists of a relatively healthy population with low MDA levels. If, rather, it consisted of diseased individuals with MDA levels in the upper part of the curve, assay replicability would be increased. Intra-assay CVs for MDA averaged 29.4%; again, this seems high, but could be acceptable if the variance across the whole sample were much higher. It is possible that our higher CVs reflect that the study population shows relatively low variance compared to within subject or even within assay variance, possibly indicating that MDA is a poor measure for this type of sample population.

For 8-OHdG, the picture is less ambiguous. The average inter-assay CV was 11.2% for the 5 calibrators. Most of the study samples fell in the most reliable part of the curve, between standards 3 and 4 (CVs of 2.5% and 3.3%, respectively), and the intra-assay CV was a respectable 6.6%.

Chapter 5: Discussion

Summary

In a sample of college-age men who report being romantically involved, relationship quality predicts oxidative stress. For individuals in relationships, greater levels of investment in relationships (estimated by INV, the composite of self and perceived partner investment) corresponded with lower levels of oxidative stress.

Discussion of the Results

This research is correlational in nature. Therefore, we are unable to infer that relationship quality causally contributes to oxidative stress. Discussed here are two plausible scenarios that could account for this association. First, a factor predisposing oxidative stress could also predispose relationship quality: e.g., men with higher general condition or quality to begin with may experience lower oxidative stress, and may also be more likely to achieve higher quality romantic relationships. Second, relationship quality could affect oxidative stress: Higher quality romantic relationships may have direct effects on health that result in lower oxidative stress levels, regardless of the men's initial quality or condition. The latter possibility is the one that drove the prediction (as noted in the introduction), but of course the former must be entertained as well. What follows is a more detailed discussion of these two possibilities.

A Factor Predisposing Oxidative Stress May Also Predispose Relationship Quality

The first scenario entails that men who have pre-existing lower genetic quality or fitness may experience greater susceptibility to oxidative stress, while men with pre-existing higher quality may have greater resistance or be generally healthier. These higher-quality men may also be more likely to achieve higher quality romantic relationships, because either a) they are generally more functional and more capable of attaining or sustaining a high quality relationship themselves, and/or, b) they are more preferable as relationship partners by females who are more

capable of doing so. This interpretation is consistent with previous suggestions that oxidative stress may be an important agent linking the expression of sexual ornaments to genetic variation in fitness-related traits (von Schantz et al 1999), and that the genes involved in immune defense and the processing of toxic compounds clearly affect health (Gonzalez & Nebert, 1990; Nebert et al., 1996; Apanius et al., 1997, Kalow, 1997).

As noted previously, four variables were identified that could reflect overall quality: morbidity, schizotypy, FA, and attractiveness. If these variables represent a predisposing factor responsible for the primary association found, they should relate to relationship quality, and to oxidative stress, regardless of relationship status. Assessment of these variables yielded inconclusive results.

In the case of morbidity, no associations were found suggesting that proneness to disease was not a significant factor in preventing men from attaining higher quality romantic relationships, and thus not responsible for the relationship between oxidative stress and relationship quality. Schizotypy associated with both oxidative stress and relationship quality in the sample of mated men, but if schizotypy were a predisposing personality trait generating the correlation between relationship quality and oxidative stress, it should have been correlated with oxidative stress in both subsamples of men, whether mated or not. The correlation was not stable across subsamples, however. This empirical pattern suggests that relationship quality may interact with schizotypy to predict oxidative stress. Schizotypy may predispose low relationship quality, which then may lead to oxidative stress, generating a correlation between schizotypy and oxidative stress, but only amongst men in relationships.

Measures of fluctuating asymmetry and attractiveness could also possibly indicate quality. However, neither FA nor attractiveness predicted relationship quality, even though they both predicted oxidative stress, both amongst mated men and in the sample at large. This pattern

does not provide substantial evidence that individual differences in FA and attractiveness account for the association between oxidative stress and relationship quality.

If the ability to resist oxidative stress is heritable and is associated with higher quality, it would be beneficial for females to detect cues informative of men's oxidative stress levels. This suggests the possibility that phenotypic cues conveying information about resistance to oxidative stress are relevant to female choice and subject to forces of sexual selection. Consistent with this possibility is the finding that oxidative stress correlated strongly with attractiveness in our sample, suggesting that females may indeed visually detect and even prefer men who have greater resistance to oxidative stress. It is unclear, however, if women are detecting cues specific to oxidative stress susceptibility or resistance, directly, or if they are detecting some general quality or fitness cues that associate with lower oxidative stress. Because relationship quality was uncorrelated with attractiveness, we did not find support for the notion that women select men for high quality relationships who already have greater resistance to oxidative stress.

Overall, while results are inconclusive, it does not appear that these four factors (morbidity, schizotypy, FA, or attractiveness) tap a predisposing factor that drives the association between oxidative stress and relationship quality.

Relationship Quality May Directly Affect Oxidative Stress

A second plausible scenario to explain the primary association found is that the experience of being in a higher quality romantic relationship may have direct effects on health, including, possibly, increased resistance to oxidative stress and its damaging effects. Generally, people who report being in higher quality romantic relationships have better psychological and physical well-being, and live longer than people not in such relationships (Chandra et al., 1983; Goodwin et al., 1987; Gordon & Rosenthal, 1995; House et al., 1988). The antioxidant defense and oxidative system in the body may represent one possible physiological mechanism by which

physical and longevity benefits accrue. Possibly, following from the original hypothesis, the experience of being in an invested, emotionally engaged relationship corresponds with a reduction in mating effort, leaving more energy to be allocated to other tasks, such as somatic investment in anti-oxidant defense systems.

If expenditure of mating effort is responsible for the primary association found, it may seem surprising that in our secondary analyses, using "relationship status" as a categorical predictor instead of "relationship quality" as a continuous one, results indicated a non-significant association between relationship status and oxidative stress. One might expect single men to categorically expend more mating effort than men in relationships. However, as emphasized in the introduction, relationship quality appears to be key to health impacts, and accordingly, this finding may reflect that men in unhappy or less established relationships are motivated to continue to expend mating effort at equivalent or even higher levels than men who are single, which might associate with higher oxidative stress levels.

If, indeed, relationship quality impacts oxidative stress directly due to reduced energy allocation to somatic repair processes, one might expect testosterone to mediate the relationship, as testosterone is commonly attributed as a hormonal mediator of mating effort in males. Consistent with such an effect, it has previously been reported that men in committed romantic relationships have lower testosterone (Booth & Dabbs, 1993; Burnham et al., 2003; Gray et al. 2002, 2004; Mazur & Michalek, 1998; McIntyre et al., 2006; Muller et al., 2008). However, we found no evidence for a testosterone-mediated effect in this sample. While this may be due to small sampling size or sampling variability, it may also suggest that testosterone's role as a mediator is muddled by variable directionality of multiple effects, as discussed earlier in the predictions section.

Another possibility is that testosterone mediates the relationship between oxidative stress and relationship quality, but in a non-linear fashion: for instance, men with very low testosterone may face challenges in attaining very invested relationships with investing partners, while men with very high testosterone may be less inclined to invest at all. However, when relationship quality and relationship quality squared were regressed on testosterone, there did not appear to be any curvilinear, quadratic relationship between relationship quality and testosterone in this sample, $F(2,38) = .148, p = .539$).

There may exist other hormonal mediators of mating effort or related phenomena not examined in this data, or not yet identified. "Attachment hormones" such as oxytocin or vasopressin could perhaps play important roles, given their prominence in mating and attachment behavior in other species, such as voles. One possibility is that affectionate behavior, and/or hormonal secretions of oxytocin or vasopressin could be mediating the relationship between oxidative stress and relationship investment.

Interpretations discussed thus far have hinged on the idea that expenditure of mating effort is key to understanding the pattern of results. An alternative could be that it's not mating effort, but a general stress effect driving results. Relationship quality may be directly related to psychosocial stress, which could affect oxidative stress: Men in higher quality relationships may experience less psychosocial stress, while those who are single or in lower quality relationships may weather stressful events with less support, and this could affect oxidative stress levels. If this were the case, one might expect cortisol to mediate the relationship between oxidative stress and relationship quality, but we found no evidence for this effect. Again, as with testosterone, interpretation of this result is inconclusive; the lack of association could stem from various sources, such as divergent directionality of multiple effects. Further, although social support has been found to associate with attenuated free cortisol levels in saliva (Kirschbaum et al., 1995)

and social support has been associated with lower stress responsiveness (Heinrichs et al., 2003), cortisol measures taken at two time points may not be valid indicators of ongoing, chronic psychosocial stress levels.

Limitations of the Study

There are two significant limitations to the current research. Firstly, as discussed above, the research is correlational in nature. Therefore, we are unable to infer that relationship quality causally contributes to oxidative stress (though, clearly, that scenario is a plausible one). Secondly, our estimate of oxidative stress is a rough one. We assessed oxidative stress through two biomarkers of oxidative stress. Some researchers note that more extensive multi-parameter batteries are possible (e.g., Tamura et al., 2006). Further, the correlation in biomarker values for our first and second urine samples was low, the reason being unclear. Additional sample times over time may improve this reliability, or sample times standardized by time of day.

This initial exploratory work used only a questionnaire methodology to assess relationship quality. More in-depth analyses of relationship quality are possible as well, via observational methods (by observing behaviors directly, rather than using paper-and-pencil measures alone) or perhaps by longitudinal, long-term tracking of relationship states.

Implications for Future Research

Future work may further examine the association between relationship quality and oxidative stress in the following ways:

First, the question of causality between relationship quality and oxidative stress may be addressed with longitudinal studies of men in various relationship states (single, newly coupled, or in an established relationship) using a repeated measures design to reveal whether or not changes in oxidative stress mirror changes in relationship quality between subjects, or within subjects over time, suggesting a direct effect of relationship quality on oxidative stress. Such a

study may also elucidate how sensitive oxidative stress levels are, temporally, to romantic interactions.

Second, future work may incorporate measures of other possible hormonal mediators of the relationship between oxidative stress and relationship quality, such as oxytocin, or in men, vasopressin.

Third, it may be useful to assess whether or not net/systemic/organismal oxidative stress levels, as estimated by biomarkers found in urine, associate with sperm quality in general, or the degree of oxidative damage to sperm. The sensitivity and specificity of oxidative stress as measured by biomarkers is unclear (Dalle-Donne et al, 2006). If oxidative stress susceptibility is generalized within an organism, men with higher susceptibility to oxidative stress may also have more oxidatively damaged sperm. Speculatively, they may even exhibit a chromosomal sex-bias in germ cells depending on the quality of the father. Future work may better assess these possibilities.

Fourth, the association we find in men between relationship quality and oxidative stress may be assessed in women, who were not included in the present study/sample. As women are thought to exert less mating effort than men, they may not pay the same cost that men do when not in highly invested relationships. For that reason, there may be no association between relationship investment and oxidative stress for women. Alternatively, because women do exert some mating effort to attract high quality males, investment in a romantic relationship could entail a reduction of mating effort in women and, consequentially, lower oxidative stress. More generally, studies incorporating females may further understanding of apparent gender disparities in effects between relationship quality and health. While marital functioning and health is clearly related in both sexes, emerging patterns suggest that the pathway between negative marital conflict behaviors and physiological functioning is stronger in women than in men, even though

marriage seems to have a greater net protective health-benefit for men, and even though marital disruption appears more detrimental to men than women (House et al., 1988; Levenson et al., 1993). It appears that relationships between negative experiences and physiological changes are typically stronger in women than men, and women's physiological changes following marital conflict show greater persistence than men's. There may also be gender differences in physiological impacts of oxidative stress. In studies of physiological correlates of marital interaction, gender differences are robust and salient (Kiecolt-Glaser & Newton, 2001), but gender has not received consistent and systematic attention regarding marital functioning and longer term physical health outcomes.

Conclusions

That good relationships and good health co-occur has been heartily observed by scientists and laypeople alike, and a vast body of scientific literature has linked positive romantic interactions with lower morbidity and mortality. The physiological mechanisms that underlie these effects, however, remain poorly understood. Meanwhile, research on oxidative stress has recently expanded, helped integrate conceptual frameworks about the ageing process, and fostered an increasingly nuanced understanding of the physiological underpinnings of senescence and disease. Oxidative stress has garnered scientific interest as a central component of these processes, and for the role it may play in trade-offs that shape the life history strategies of organisms. The finding that oxidative stress is negatively associated with relationship quality ties these two bodies of research together, and presents intriguing avenues for further research into the physiological pathways by which psychological effects may impact human health, or vice versa. Further empirical work may lead to further insight about how human life strategies are influenced by oxidative stress via processes of selection, and may implicate a physiological mechanism by which romance directly impacts human health.

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Appendix A: Relationship Quality Scale

1. Partner Specific Inventory Scale:

Self-investment component: Do you do these things with your partner?

1. I make and discuss plans for our future
2. I avoid doing things with my partner's family
3. I want to have sex with my partner
4. I pay for our evening entertainment
5. I refer to my partner publicly as my girlfriend
6. I flirt with other women in front of my partner
7. I start arguments with my partner over trivial issues
8. I take my partner out to eat at restaurants
9. I bring my partner to my family gatherings
10. I talk about the attractiveness of other women in my partner's presence
11. I make sure my partner doesn't have to go out alone at night
12. I make a special effort to spend time with my partner
13. I desert my partner at parties
14. I ask for my partner's opinion about things
15. I lie to my partner about important things
16. I share my feelings with my partner
17. I comfort my partner when she is distressed
18. I break plans with my partner to go out with my friends
19. I display concern for my partner's problems

20. I tell my partner little lies then try to wiggle out of them
21. I try to please my partner sexually
22. I ignore my partner in social settings
23. I escort my partner in potentially dangerous situations (such as walking her home at night)
24. I try to deceive my partner
25. I trust my partner with secrets that I do not want anyone else to know
26. I am willing and able to express my thoughts to my partner
27. I buy my partner gifts
28. I have sexual intercourse with my partner
29. I prefer to spend my free time with my friends rather than with my partner
30. I pretend in public that my partner and I are just friends
31. I talk in the inclusive “we”
32. I look at other women when we go out together
33. I cancel dates with my partner at the last minute
34. I don't pay attention to my partner when we are around my friends
35. I refuse to have sex with my partner

Partner-investment component: Does your partner do these things with you?

1. She makes and discusses plans for our future
2. She avoids doing things with my partner's family
3. She wants to have sex with me
4. She pays for our evening entertainment
5. She refers to me publicly as her boyfriend
6. She flirts with other men in front of me

7. She starts arguments with me over trivial issues
8. She takes me out to eat at restaurants
9. She brings me to her family gatherings
10. She talks about the attractiveness of other men in my presence
11. She makes sure I don't have to go out alone at night
12. She makes a special effort to spend time with me
13. She deserts me at parties
14. She asks for my opinion about things
15. She lies to me about important things
16. She shares her feelings with me
17. She comforts me when I am distressed
18. She breaks plans with me to go out with her friends
19. She displays concern for my problems
20. She tells me little lies then tries to wiggle out of them
21. She tries to please me sexually
22. She ignores me in social settings
23. She escorts me in potentially dangerous situations (such as walking me home at night)
24. She tries to deceive me
25. She trusts me with secrets that she does not want anyone else to know
26. She is willing and able to express her thoughts to me
27. She buys me gifts
28. She has sexual intercourse with me
29. She prefers to spend her free time with her friends rather than with me
30. She pretends in public that she and I are just friends

31. She talks in the inclusive “we”
32. She looks at other men when we go out together
33. She cancels dates with me at the last minute
34. She doesn’t pay attention to me when we are around my friends
35. She refuses to have sex with me

2. Relationship status question:

Current relationship status (Check all that apply)

- married
- married but separated
- divorced
- engaged to be married
- not current married, but cohabiting with a romantic partner
- dating one person exclusively
- dating multiple persons
- not currently dating

Appendix B: Perceived Vulnerability to Disease Scale

1. I am comfortable sharing a water bottle with a friend
2. I suffer quite intense symptoms when I do get sick
3. It really bothers me when people sneeze without covering their mouths
4. I don't like to write with a pencil that someone else has obviously chewed on
5. My past experiences tell me that I'm not likely to get sick even when my friends are sick
6. I prefer to wash my hands pretty soon after shaking someone's hand
7. I dislike wearing used clothes because you don't know what the past person who wore it was like
8. If an illness is "going around," I will get it
9. I don't worry about contamination if I touch an animal
10. In general, I am very susceptible to colds, flu, and other infectious diseases
11. I think day care centers are breeding grounds for bacteria and germs
12. I am more likely than other people around me to catch an infectious disease
13. My hands do not feel dirty after touching money
14. I am unlikely to catch cold, flu, or other illnesses, even if it is going around
15. It does not make me anxious to be around sick people
16. My immune system protects me from most illnesses that other people get
17. I avoid using public telephones because of the risk that I may catch something from the previous user
18. I have a history of susceptibility to infectious diseases
19. I thoroughly wash cuts and scrapes to avoid infections

Appendix C: Schizotypy Scale

Note: MIS items are indicated with two stars (**)

STS items are indicated with three stars (***)

SAS items are indicated with no stars

- **1. Some people can make me aware of them just by thinking about me.
- 2. Having close friends is not as important as many people say.
- **3. I have had the momentary feeling that I might not be human.
- 4. I attach very little importance to having close friends.
- 5. I prefer watching television to going out with other people.
- **6. I have sometimes been fearful of stepping on sidewalk cracks.
- 7. A car ride is much more enjoyable if someone is with me.
- **8. I think I could learn to read other's minds if I wanted to.
- 9. I like to make long distance phone calls to friends and relatives.
- 10. Playing with children is a real chore.
- **11. Horoscopes are right too often for it to be a coincidence.
- 12. I have always enjoyed looking at photographs of friends.
- **13. Things sometimes seem to be in different places when I get home, even though no one
been there.
- **14. Numbers like 13 and 7 have no special powers.
- 15. Although there are things that I enjoy doing by myself, I usually seem to have more fun when
I do things with other people.
- 16. I sometimes become deeply attached to people I spend a lot of time with.

- **17. I have occasionally had the silly feeling that a TV or radiobroadcaster knew I was listening to him.
18. People sometimes think I am shy when I really just want to be left alone.
- **19. I have worried that people on other planets may be influencing what happens to me on earth.
20. When things are going really good for my close friends, it makes me feel good too.
21. When someone close to me is depressed, it brings me down also.
- **22. The government refused to tell us the truth about flying saucers.
23. My emotional responses seem very different from those of other people.
- **24. I have felt that there were messages for me in the way things were arranged, like in a store window.
25. When I am home alone, I often resent people telephoning me or knocking on my door.
26. Just being with friends can make me feel really good.
- **27. I have never doubted that my dreams are the product of my own mind.
28. When things are bothering me, I like to talk to other people about it.
- **29. Good luck charms don't work.
30. I prefer hobbies and leisure activities that do not involve other people.
31. It's fun to sing with other people.
- **32. I have noticed sounds on my records that are not there at other times.
33. Knowing that I have friends who care about me gives me a sense of security.
- **34. The hand motions that strangers make seem to influence me at times.
35. When I move to a new city, I feel a strong need to make new friends.
36. People are usually better off if they stay aloof from emotional involvements with most others.

- **37. I almost never dream about things before they happen.
38. Although I know I should have affection for certain people, I don't really feel it.
- **39. I have had a momentary feeling that someone's place has been taken by a look-alike.
40. People often expect me to spend more time talking with them than I would like.
41. I feel pleased and gratified as I learn more and more about the emotional life of my friends.
- **42. It is not possible to harm others merely by thinking bad thoughts about them.
43. When others try to tell me about their problems and hang-ups, I usually listen with interest and attention.
- **44. I have sometimes sensed an evil presence around me, although I could not see it.
- **45. I sometimes have a feeling of gaining or losing energy when certain people look at me or touch me.
46. I never really had close friends in high school.
47. I am usually content to just sit alone, thinking and daydreaming.
- **48. I have sometimes had the passing thought that strangers are in love with me.
49. I'm much too independent to really get involved with other people.
- **50. I have never had the feeling that certain thoughts of mine really belonged to someone else.
51. There are few things more tiring than to have a long, personal discussion with someone.
52. It made me sad to see all my high school friends go their separate ways when high school was over.
- **53. When introduced to strangers, I rarely wonder whether I have known them before.
54. I have often found it hard to resist talking to a good friend, even when I have other things to do.
- **55. If reincarnation were true, it would explain some unusual experiences I have had.
56. Making new friends isn't worth the energy it takes.

57. There are things that are more important to me than privacy.
- **58. People often behave so strangely that one often wonders if they are part of an experiment.
59. People who try to get to know me better usually give up after a while.
- **60. At times I perform certain little rituals to ward off negative influences.
61. I could be happy living all alone in a cabin in the woods or mountains.
62. If given the choice, I would much rather be with others than be alone.
- **63. I have felt that I might cause something to happen just by thinking too much about it.
64. I find that people too often assume that their daily activities and opinions will be interesting.
- **65. I have wondered whether the spirits of the dead can influence the living.
66. I don't really feel very close to my friends.
67. My relationships with other people never really get very intense.
- **68. At times I have felt that a professor's lecture was meant especially for me.
69. In many ways, I prefer the company of pets to the company of people.
- **70. I have sometimes felt that strangers were reading my mind.
- ***71. I have not lived the right kind of life.
- ***72. I have been disappointed in love.
- ***73. My sex life is satisfactory.
- ***74. I am more sensitive than most other people.
- ***75. I am sure I am being talked about.
- ***76. I usually work things out for myself rather than get someone to show me how.
- ***77. I enjoy many kinds of play and recreation.

Appendix D: Smoking and exposure to toxins items

Do you smoke tobacco?

_____ Yes, 1 to 5 cigarettes a day

_____ Yes, 6 to 10 cigarettes a day

_____ Yes, more than 11 cigarettes a day

_____ No, I have stopped smoking (I previously smoked regularly for ____ years)

_____ No, I have never smoked

Do you work in an environment that exposes you to toxins on a regular basis? (This could be fumes from car exhaust, paint, pesticides, asbestos, radiation, mercury, etc.)

_____ Yes (please explain here: _____)

_____ No

Have you been exposed to any environmental toxins within the last 2 days. (This could be fumes from car exhaust, paint, pesticides, asbestos, radiation, mercury, etc.)

_____ Yes (please explain here: _____)

_____ No