17β-ESTRADIOL AND TESTOSTERONE LEVELS IN AXILLARY PERSPIRATION OF MEN:   
ENVIRONMENTAL FACTORS, INTER- AND INTRA-INDIVIDUAL VARIATION

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A Thesis Submitted to the School of Graduate Studies in Partial Fulfilment of the Requirements for the Degree Master of Science

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TITLE: 17β-Estradiol and testosterone levels in axillary perspiration of men: Environmental factors, inter- and intra-individual variation

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**Abstract**

In rodents, there is accumulating evidence that sex steroids, particularly estradiol (E2), have pheromonal properties. Male mice actively direct their urine, which contains these hormones in abundance, at proximal females. Radiolabelled E2 injected into male mice has subsequently been found in females that cohabited with these males for a few days, especially in these females’ reproductive systems and brains. Little is known about the potential pheromonal properties of E2 and other sex steroids such as testosterone (T) in humans, however. Previous work from this laboratory found remarkable inter-individual variation in levels of E2 and T in axillary perspiration of men, and levels of E2 and T in the axilla correlated very poorly with levels of the same hormones in other substrates. The axilla has unique histological properties which may allow it to synthesize these hormones de novo. This project aimed to investigate inter- and intra-individual variation in levels of E2 and T in the axillary perspiration of men, and to assess the relationships of various environmental factors to these hormones. Eighty-one males were recruited from the David Braley Athletic Centre at McMaster University and asked to donate four perspiration samples approximately 1-2 weeks apart. Participants were randomly assigned to have the first two sessions conducted by a female researcher and the last two conducted by a male researcher, or vice versa. Participants also filled out, during the first session, a questionnaire assessing various environmental factors which we suspected might be related to axillary sex steroid levels. These factors included dietary phytoestrogen consumption, stress level, relationship status, and recent sexual contact. E2 and T were measured using enzyme-linked immunosorbent assay (ELISA). Overall, levels of both axillary E2 and T were fairly stable within an individual but ranged widely among individuals. E2 and T correlated very strongly with one another (i.e. from the same individual from the same session). A composite score indicating recent romantic/sexual contact with females correlated significantly with an individual’s average axillary E2, but this score did not correlate significantly with average T. However, when the samples conducted by the female researcher and male researcher were considered separately, the composite score correlated significantly with E2 in measures taken by the female researcher, but not in those taken by the male researcher. There was a similar trend, albeit non-significant, between this composite and T in samples taken by the female researcher. A multiple regression analysis was performed using age, a phytoestrogen composite score, a stress composite score, an exercise score, a homosexuality composite score, a composite score of relations with females, and a birth control pill exposure composite score as predictors of axillary E2. This did not show significant prediction when all measures of E2 were considered, but it was significant considering only the measures conducted by the female researcher. Overall, the intra-individual stability noted in levels of axillary E2 and T in men suggests stable inter-individual differences, possibly subserved by genetic factors. However, many other factors, including environmental conditions consistent throughout the duration of this project, may also affect axillary sex steroids. It is possible that contact with proximal females promotes an increase in levels of axillary E2 and T in men. Since these hormones are readily absorbed transdermally, the potential for E2 and T to be transferred via perspiration is worth further investigation.

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**List of Abbreviations**

Estradiol E2

Estriol E3

Estrone E1

Testosterone T

Estrogen Receptor ER

Androgen Receptor AR

Progesterone P4

IGF-1 Insulin-like growth factor 1

Sex-hormone binding globulin SHBG

Antiperspirant Antp

**Declaration of Academic Achievement**

The contents of this thesis were contributed to by Brittney Elliott, in partial fulfillment of the requirements for the degree Master of Science, who consulted with Dr. Denys deCatanzaro. All experiments were designed by Dr. deCatanzaro and Brittney Elliott, and were conducted by Brittney Elliott and Ellis Freedman.

1.0. Introduction: 17β-Estradiol and Testosterone as Potential Pheromones in Humans   
  
**1.1. Overview**  
  
 Steroid hormones are a class of molecules synthesized from cholesterol. This thesis focuses on 17β-estradiol (E2), an estrogen, and on testosterone (T), an androgen. In laboratory rodents, evidence indicates that these sex steroids can be transmitted between conspecifics and that this transmission may cause pheromonal effects, and these effects may also occur in other mammals (see review by deCatanzaro, 2015). However, it is currently unclear whether similar mechanisms exist in humans. Previous research from this lab has found extraordinary inter-individual variation in levels of E2 and T in axillary perspiration of young adult males (Muir et al., 2008). This phenomenon appears to be unique to axillary perspiration, i.e. men with exceptionally high axillary levels of E2 and T do not show similar levels in perspiration from their face. The present research was undertaken to examine intra-individual stability in levels of E2 and T in young adult males' axillary perspiration, with the ultimate goal of understanding the extent to which genetic vs. environmental factors contribute to the extraordinary inter-individual variation previously found. This question is of interest because axillary perspiration provides a potential vector for the pheromonal transmission of these sex steroids between humans.   
  
**1.2. Estradiol and Testosterone: Synthesis and Mechanisms of Action**  
  
 Sex steroids (estrogens and androgens) are synthesized from cholesterol in the gonads, namely the testes in males and the ovaries in females. In many mammals including humans, the adrenal cortex also produces sex steroids, and there is evidence that this includes laboratory rodents (e.g. Thorpe et al., 2014). Essentially, the adrenal cortex and the gonads are both managed by the pituitary gland, which is itself controlled by the hypothalamus.  
 Steroid hormones are generally slow-acting messengers, exerting their effects over a period of hours or days by altering gene expression. Their lipophilic nature, small molecular size and polarity allow them to easily cross cell membranes. They travel through the bloodstream bound to various classes of protein carriers; for example, sex hormone-binding globulin (SHBG) binds approximately 98% of serum E2 (see review by Alonso and Rosenfield, 2002). Most commonly, sex steroids exert their effects by travelling into the cytoplasm and binding to receptors inside of the cell. The two established genomic (intracellular) estrogen receptors (ER) are the proteins ERα and ERβ, which, in their classical mode of operation, dimerize when bound to E2. These E2:ER complexes translocate to the nucleus and recruit the other components of the transcription machinery, including coactivators and/or corepressors. This machinery interacts with estrogen response elements (ERE) within the promotor regions of target genes, thus altering gene expression, generally by stimulating transcription (reviewed by Nilsson et al., 2001). In addition, non-genomic, rapid actions of estrogens have recently been reported. More than one membrane-bound ER responsible for these actions have been discovered. These include subpopulations of the classical ERα and ERβ proteins (mERα and mERβ), as well as a G-protein coupled estrogen receptor (GPER; see review by Barton, 2012). One example of the rapid effects believed to be a response to estrogen is the synthesis of nitric oxide in vascular endothelial cells, which causes vasodilation and increases blood flow (Barton, 2012).   
 Across mammalian species, estrogen receptors are found in the reproductive organs (uterus and ovaries) of females (Kuiper et al., 1997). They are also present in regions of the central nervous system (CNS), including the limbic system and hypothalamus (Pfaff, 1980; Simerly et al*.*, 1990), which have been implicated in many aspects of motivation, emotion and behaviour. ER are present throughout the body in both sexes, including some existence in the kidneys, bladder, lungs, and olfactory bulbs, among other tissues (Kuiper et al., 1997).   
 In addition to E2, there are two other major estrogens: estrone (E1, the estrogen which increases in postmenopausal women), and estriol (E3, the weakest of these three major estrogens and the one which increases during human pregnancy). This work focuses on E2 because it is the most potent estrogen, binding both ERα and ERβ more strongly than E1 or E3 (Kuiper et al., 1997), and because increasing evidence implicates E2 as an important chemosignal or pheromone in certain mammals (e.g. deCatanzaro, 2015).  
 Androgens are precursors to estrogens, and are also synthesized from cholesterol. Aromatase is an enzyme which readily converts androstenedione and T into estrone and E2, respectively. Similarly to the estrogens, T exerts its affects via a genomic androgen receptor (AR), but can also elicit rapid, non-genomic effects (see review by McEwan, 2004). Another important androgen (though in many effects it is not as potent as T) is dihydrotestosterone (DHT), which also binds the AR (McEwan, 2004). The AR binds DNA as a homodimer, recruiting other components of the transcription machinery and thus altering gene expression (McEwan, 2004). The AR, like the ER, is found in many mammalian tissues; in humans, it can be found in the prostate, testes, sweat glands of the skin, and in the liver, to name a few (Kimura et al., 1993).  
  
**1.3. Estradiol and Testosterone: Roles in Reproductive Physiology and Behaviour**  
  
1.3.1. E2, T, and Fertility in Mammals  
 Sex steroids, particularly E2, are essential for reproductive maturation, sexual receptivity, and reproduction in mammals. In female mammals in general, endogenous estrogens are essential for normal puberty, including growth of the reproductive tissues (reviewed by Alonso and Rosenfield, 2002); for example, E2 promotes DNA synthesis and cell proliferation in the mouse uterus (Ogasawara et al., 1983). E2 regulates growth hormone and insulin-like growth factor 1 (IGF-1) activity (reviewed by Leung et al., 2004), and local IGF-1 activity mediates uterine growth in response to E2 (Sato et al., 2002). Although other estrogens can promote maturation of the reproductive organs, estradiol does so most dramatically. For example, Anderson et al. (1975) found that E3 did not cause significant growth of rat uteri after 24 hours, whereas E2 caused a notable increase in dry weight. The authors suggest that this is due to a much longer time of residence of the E2:ER complex vs. the E3:ER complex in the cell nucleus.  
 Most female mammals experience what is called an estrous cycle. The analogous cycle in humans and some other primates is called the menstrual cycle, which involves many of the same dynamics but also includes the loss of the uterine lining during menstruation. Female rats come into estrus (their most fertile period) approximately every 5 days, and are only receptive to advances of males during this time. Actions of E2 at the hypothalamus are critical for this sexual receptivity (e.g. Pfaff, 1980). Female rats that have had their ovaries removed no longer display receptivity, unless they are induced to do so with injections of E2 and progesterone (P4; Green et al., 1967). Estradiol affects women's sexual behaviour as well, though it is likely not the only hormone that does so. As mentioned, estrogen receptors in the hypothalamus are important for sexual receptivity in mammals (e.g. Pfaff, 1980). In primates, the adrenal glands appear to control the female sexual response more than do the ovaries; women who have had their adrenal glands removed show reduced sexual interest (Waxenberg et al., 1959). Whereas E2 and P4 control the female sexual response in simpler mammals such as rodents, there is increasing evidence that androgens, particularly T, are also important for sexual behaviour in some female primates. In particular, Guay and Jacobson (2002) found that 70% of women complaining of decreased sexual desire, in a group consisting of both premenopausal and postmenopausal women, had lower than normal levels of total T, free T, and dehydroepiandrosterone-sulfate (DHEA-S, one of the precursors to T). There is some evidence that T can increase libido in postmenopausal women with decreased sexual interest (reviewed by Basson, 2010). Barton et al. (2007) suggest that the effect of T may be mediated by levels of estrogens. They found that female cancer survivors given transdermal T did not experience greater libido than survivors given placebo, and they assert that this may be because their study cohort was estrogen-depleted, but further studies are needed to explore this. Davis et al. (2006) administered transdermal T to postmenopausal women who were already using transdermal estrogen therapy, but they also gave participants an aromatase inhibitor. They found that increases in total and free T were associated with improved sexual satisfaction, well-being, and mood, and aromatase inhibition did not affect these outcomes. Therefore, if these effects of T are mediated by estrogens, it is unlikely that it is conversion of T to E2 that is responsible for the mediation.  
 Although the roles of the three major estrogens in human pregnancy have not been entirely discovered, it is thought that they, along with other hormones, contribute to the regulation of events leading up to birth. One study found that women who delivered preterm had a higher E2:P4 ratio in both their amniotic fluid and plasma (Mazor et al., 1994). Urinary E3, which is much weaker than E2, increases 1000-fold in pregnant women, and thus likely plays an important role in reproduction. It is possible that this elevated E3 may saturate estrogen receptors, protecting the fetus from more potent estrogens which could disrupt pregnancy even in minute doses. Indeed, just 37 ng of E2 given subcutaneously and daily to mice on gestation days 1-5 can terminate pregnancy, and this is much lower than the doses of E1, E3, or T required for implantation failure (deCatanzaro et al., 1991, 2001).   
 Estradiol affects the rate of passage of fertilized ova through the fallopian tubes (e.g. Ortiz et al., 1979) and has major influences over the receptivity of the uterus to blastocysts, determining the duration of the implantation window (Ma et al., 2003). Paradoxically, estrogens can cause pregnancy termination, but are also crucial for maintaining pregnancy, and their effect appears to depend on both their concentration and timing. When the oocyte is first fertilized, E2 is conducive to its implantation, because E2 promotes the production of uterine epithelial cells, as well as tissue edema, induction of P4 (which promotes uterine and endometrial growth) receptors, and arrival of leukocytes (Hunt et al., 2000; Tibbetts et al., 1999). However, if E2 is elevated even minutely above optimal levels, this can prevent blastocyst implantation altogether (deCatanzaro et al., 1991, 2001; Ma et al., 2003). One possible explanation is that low doses of exogenous E2 can hasten the transport of the embryo from the oviduct to the uterus; at the wrong time, this would cause premature arrival of the embryo at the uterus, resulting in its removal through the vagina (Ortiz et al., 1979). Other effects of E2 include an induction of fluid flow into the uterine lumen, preventing it from closing in around blastocysts, and a suppression of e‑cadherin, a molecule that promotes adhesion of the blastocyst to the uterine epithelium (Rajabi et al., 2014; Potter et al., 1996). Given the dramatic influence that E2 has on reproductive physiology and behaviour, transmission of sufficient concentrations of E2 between humans may affect reproduction.   
  
1.3.2. Established Pheromonal Effects in Mammals   
 There are several pheromonal effects observable across mammals in which estrogens appear to play a crucial role, and given the highly conserved nature of steroid dynamics, there is reason to believe that E2 may have pheromonal properties in humans (see review by deCatanzaro, 2015). This section will focus on three mammalian pheromonal effects: the Vandenbergh effect, the Bruce effect, and the Whitten effect.   
 The Vandenbergh effect is the hastening of reproductive maturation of juvenile females by exposure to adult male conspecifics (Vandenbergh, 1967). The Vandenbergh effect has been studied most thoroughly in mice (e.g. Vandenbergh, 1967), but it has been documented in many mammals, as reviewed by deCatanzaro (2015). These mammals include hamsters (Reasner and Johnston, 1988), opossums (Harder and Jackson, 2003), voles (Hasler and Nalbandov 1974), and cattle (Izard and Vandenbergh, 1982). The uteri of juvenile female mice can be enlarged by only 36 hours of male exposure (Bronson and Stetson, 1973). Plasma E2 and P4 both increase dramatically after male exposure (Bronson and Desjardins, 1974). Transfer of E2 from males to females may be sufficient to cause the Vandenbergh effect, because two injections of 100 ng E2-benzoate to immature females, on successive days, induce vaginal opening similar to that caused by male exposure (Bronson, 1975).   
 The Bruce effect is the termination of early gestation in mammals by the introduction of non-sire males (Bruce, 1960). This effect has also been documented in several mammals, and there is strong evidence that E2 transmission plays a crucial role, at least in mice (deCatanzaro, 2015). Giving females low doses of E2 can mimic both the Vandenbergh (Bronson, 1975) and Bruce (deCatanzaro et al., 1991) effects. Castrating a male mouse reduces his ability to induce both the Vandenbergh and Bruce effects, and injecting a castrated mouse with androgens (Bruce, 1965; deCatanzaro and Storey, 1989) *or* estrogens (deCatanzaro et al., 1995a; Thorpe & deCatanzaro, 2012) restores this ability. Since T is a direct precursor of E2, it is plausible that exogenous T injected into a male is converted to E2, and that this is responsible for the restored ability to induce the Vandenbergh and Bruce effects. In addition, most inseminated females exposed to novel males retain their pregnancies if they are given estrogen antibodies, but most lose their pregnancies without these antibodies (deCatanzaro et al., 1995b). One possible factor contributing to this pregnancy loss could be the effects that E2 has of the timing of embryo transport, as mentioned. In addition, females exposed to males demonstrate suppression of e‑cadherin, an adhesion molecule that assists with blastocyst adhesion to the uterine wall (Rajabi et al., 2014). This male exposure also reduces uterine closure (i.e. increases luminal space) around blastocysts (Rajabi et al, 2014). All of these factors decrease the likelihood of successful implantation.   
 The Whitten effect describes the suppression of the estrous cycle in female mice housed together, as well as the promotion of estrous cycling in anestrous females exposed to males (Whitten, 1956, 1958). Exposure to male urine (in the absence of the male) is sufficient to mimic this effect (Marsden and Bronson, 1964), as is exposure to androgen-treated spayed female mice (Bronson and Whitten, 1968). It is possible that exposure to sex steroids from male conspecifics promotes ovulation, and that similar effects may exist in humans.   
   
1.3.3. Potential Pheromonal Effects of Sex Steroids in Humans  
 Female mammals undergo cyclical hormonal fluctuations that are correlated with behavioural changes. Estradiol and P4, along with follicle stimulating hormone (FSH) and luteinizing hormone (LH), regulate the menstrual cycle. It is possible that the transmission of axillary sex steroids from men to women may promote fertility by affecting age of reproductive maturation and by promoting ovulation and/or regulating the menstrual cycle.   
 Average ('normal') menstrual cycle lengths are more frequent in women who are regularly sexually active (Cutler et al., 1979, 1985). Given the high concentration of sex steroids in certain males' axillary perspiration (Muir et al., 2008), hormone transmission from male to female via sweat may help to explain this phenomenon. In fact, Cutler et al. (1986) found that application of extracts of male axillary perspiration for 12.5-14.5 weeks reduces the frequency of aberrant cycle lengths and reduces variability in cycle lengths. Preti et al. (2003) also found that male human axillary secretions advance the onset of female participants' next LH peaks, as well as reduce self-reported tension and increase self-reported relaxation. It should be noted that the pheromones of women may affect each other as well. For example, compounds from the underarms of females have been noted to affect menstrual cycle length in other females (Stern and McClintock, 1998). It is possible that the sex steroids within axillary perspiration are responsible for these phenomena.   
 Whitten (1956) first noted that female and male mice housed together mated fewer times than expected during the first two days, and more times than expected on the third day. However, if the males were housed beside the females for the first two days, more matings occurred the first day they were housed together. Whitten (1956) suggested the timing of the females' estrous cycles was altered by the presence of males. Giving mice low doses of E2 can mimic this male-induced estrus and ovulation (e.g. Pfaff, 1980). In humans, there is also evidence that male exposure induces ovulation. One study observed more frequent ovulation in women who spent at least two nights (in a forty day period) with men than in women who spent zero or one of those nights with men (Veith et al., 1983). It is possible that pheromonal effects are responsible for these observations.   
 Recent research has examined whether the Vandenbergh effect exists in humans. Greater pre-pubertal increases in serum E2 are associated with earlier onset of menses and more rapid pubertal development in girls (Apter and Vihko, 1985), so it is possible that exogenous estrogens (e.g. those transferred from another individual) could affect pubertal development. However, these girls with earlier menarche generally have higher E2 after puberty as well, and it is possible that these effects are caused by differences in endogenous estrogens rather than exogenous estrogens. Furthermore, it is difficult to experimentally test (i.e. in a controlled manner) whether exposure to novel males hastens reproductive maturation in humans. However, one question which can be assessed is whether stepfather presence might affect girls' age at puberty. Whether this happens is unclear, but it is certainly possible. One study—which used self-report measures and relied on participants' memory of their adolescence—found that father absence predicted an earlier age of puberty in girls, but that stepfather presence was not a predictor (Bogaert, 2005). Another study, which used established scales to assess their participants once in the sixth grade and then again a year later, found that stepfather presence was an even stronger predictor of early menarche than father absence (Ellis and Garber, 2000).   
 It should be noted that other factors may be important for mediating the roles of sex steroids in reproductive maturation. For example, kisspeptin, a protein produced by hypothalamic neurons that stimulates GnRH production, is believed to affect pubertal onset via the regulation of the hypothalamic-pituitary-gonadal axis (see review by Hameed et al., 2011).  Evidence suggests that kisspeptin’s stimulation of GnRH is in turn stimulated by E2 (Pielecka-Fortuna et al., 2008).  
   
1.3.4. Testosterone and Estradiol Dynamics in Males  
 Testosterone, particularly during embryonic development, is considered essential for proper development of the male reproductive organs and masculinization of the brain. However, it should be noted that estrogens are also crucial for this development. Evidence suggests very strongly that E2 is formed in the brain via aromatization of T, and that many of its effects on behaviour are dependent upon this conversion (Gorski, 1993, Reddy et al., 1974). Injections of estrogens in rats in the first 10 days of life masculinize later sexual behaviour at lower concentrations than do injections of androgens (Booth, 1977; Feder and Whalen, 1965). Overall, estrogens are crucial not just for the typical female behavioural profile, but also for the typical male behavioural profile.  
 Men do not exhibit monthly hormonal cycles as women do, but their steroid levels do vary. For example, levels of T in men are highest in the morning and decline throughout the day, restoring during the night (Reinberg et al., 1975). Testosterone and E2 also exhibit changes with age; from adolescence to old age, both of these hormones tend to decline in concentration (Ferrini et al., 1998). Certain environmental factors, such as stress, have also been reported to affect reproductive physiology in men, for example by depressing T (see review by McGrady, 1984).   
 In mice, exposure to females can increase creatinine-adjusted urinary T, as well as E2 (deCatanzaro et al., 2009). In men shown sexually explicit photographs, serum T has been found to be positively correlated with viewing time (Rupp and Wallen, 2007). If viewing time indicates sexual interest and/or arousal, one possible interpretation is that this interest increases levels of T. Therefore, men exposed to women, especially those they are sexually attracted to (e.g. their girlfriends if they are in a relationship), could demonstrate higher overall levels of T.  
 If exposure to women can increase sex steroids in men, it may alter behaviour in a way that promotes competition for mate access. The challenge hypothesis posits that males' T levels increase during inter-male competition, e.g. for territory or for mate access, and that this promotes aggression (e.g. Wingfield et al., 1990). There is evidence that in rodents, T modulates vasopressin receptors in the hypothalamus, which facilitates aggression (Delville et al., 1996). In addition, if levels of sex steroids increase during female exposure, these hormones may also be excreted and enter a female's system to affect her physiology and behaviour.  
  
1.3.5. Sex Steroids in Human Excretions  
 Muir et al. (2008) measured levels of E2, T, and P4 in human urine, saliva, axillary perspiration, and facial perspiration. Men's axillary perspiration showed higher levels of steroids than other substrates from men or any substrate from women, preadolescent boys, or preadolescent girls. Levels of T were, on average, 90-fold higher, and levels of E2 were 45-fold higher, in men's axillary vs. facial perspiration. Correlations of the same hormone between substrates were generally quite low. Sex steroids in men's axillary perspiration were very variable across individuals; T ranged from 18 to 1671 ng/mL and E2 ranged from 2 to 397 ng/mL. Axillary E2 correlated significantly with axillary T and axillary P4, which is unsurprising given that T and P4 are direct precursors of E2. Neither T nor E2 in perspiration was related to homosexuality.  
 The causes of this extreme inter-individual variation in men's axillary sex steroids could be genetic, environmental, or both. It is possible that producing high levels of these steroids in the axilla confers some adaptive advantage via a pheromonal response in mates or potential mates. It is also possible that the production of these steroids is a response to environmental triggers, such as proximate conspecifics of the opposite sex. Lastly, it is also possible that this is a vestigial feature and that the excretion of steroids, while important in other mammals such as mice, does not hold adaptive significance in humans. However, there are cells in the human underarm that appear to contain enzymes to modify steroids (Barth and Kealey, 1991; Takayasu et al., 1980), and such cells can possibly synthesize sex steroids de novo (Rothardt and Beier, 2001; Zouboulis et al., 2007), suggesting these hormones may be actively synthesized for an adaptive purpose.  
  
**1.4. The Functionality of the Human VNO, and a Broader Definition of Pheromone**  
  
1.4.1. The Human VNO and Human Pheromones  
 A vomeronasal organ (VNO), or Jacobson's organ, is an olfactory sense organ found in many animals that is used mainly to detect pheromones. Neurons in the VNO have axons that project to the accessory olfactory bulb and ultimately to the hypothalamus, allowing chemosignals to affect physiology and behaviour via the hypothalamic-pituitary axis (see reviews by Dulac and Torello, 2003; Kohl, 2001).  
 The potential action of pheromones in humans through a VNO is an unresolved issue (see review by Dulac and Torello, 2003). Humans have a VNO (or VNO-like structure), and some cells in the adult human vomeronasal pit are structured such that they may function as chemoreceptors (see review by Monti-Bloch et al., 1998). However, the neuronal axons of such cells have not been shown to connect to the brain (Dulac and Torello, 2003). In addition, cells in the vomeronasal pit do not stain positive for either the olfactory marker protein (OMP, which is generally expressed in olfactory chemoreceptors) or S-100 (which is generally expressed by glial cells surrounding the vomeronasal nerve bundles of other species; Trotier et al., 2000). Another discovery casting doubt on the function of the human VNO is that the gene that codes for the ion channel TRP2, which is essential for VNO function in mice, is a pseudogene in humans (Dulac and Torello, 2003).   
 Many pheromones, or potential pheromones, are airborne chemosignals which are transmitted through a VNO to affect the physiology and/or behaviour of conspecifics. It should be noted, however, that a VNO is not essential for communication via airborne pheromones. Some (e.g. Wysocki and Preti, 2004) argue that pheromonal responses in humans could be mediated by the olfactory neuroepithelium rather than the VNO. Chemosignals may be transmitted through other routes as well. For example, male mice actively direct their urine at females, and hormones in the urine may thusly be transmitted to females (deCatanzaro et al., 2009). Perspiration may offer another mode of pheromonal transmission for various species. For the purposes of this work, the term pheromone will be used to describe any chemical which can act as a chemosignal to alter the physiology and/or behaviour of conspecifics, regardless of the mode of transmission.   
 There is some evidence of pheromonal communication in humans, even though it is unclear whether this communication is dependent on a functional VNO. For example, specific androgen-and-estrogen-like compounds found in the underarm have been implicated as human pheromones. The smelling of these compounds can cause sex-specific activation in regions of the hypothalamus (Savic et al., 2001), increase skin conductance and alter mood (Jacob et al., 2001), and affect overall physiological arousal (Bensafi et al., 2003), suggesting that these compounds may act as chemosignals in humans. In addition, it has been suggested that females may be able to regulate each other's menstrual cycles, as some studies have found that the menstrual cycles of women living in close proximity tend towards synchronizing (e.g. McClintock, 1971). Compounds from the underarms of women, when administered to recipient females, have been found to alter the timing of the menstrual cycle (Stern and McClintock, 1998). However, errors in McClintock's model have been noted that would systematically increase the probability of finding menstrual synchrony in a sample (see review by Wilson, 1992). Overall, the existence of pheromonal communication between humans is probable, though exactly which chemicals are chemosignals, and the exact mechanisms through which they act, have not been fully elucidated.   
   
1.4.2. Estradiol Transmission in Nonhuman Animals   
 As reviewed by deCatanzaro (2015), steroid hormones are lipophilic and have low molecular mass, allowing them to readily enter various bodily excretions, and to enter circulation after exposure. Transdermal absorption has been demonstrated for E2, P4, and T (e.g. Guzzo et al., 2012). These hormones could potentially be absorbed nasally as well, due to the large surface area, absorbent endothelium, and highly vascularized mucosa of the nasal membrane (Türker et al. 2004). As deCatanzaro (2015) notes, there is also evidence that lipophilic molecules may pass straight into cerebrospinal fluid from the nasal cavity via the cribriform plate, which means they may be able to directly reach the brain.   
 Evidence suggests that steroid hormones do indeed transfer between conspecifics, at least in mice; tritiated E2 (3H-E2) enters the system of female mice when applied to the nasal area, in fact much more so than 3H-T or 3H-P4 (Guzzo et al., 2012). 3H-E2 injected into male mice can be found days later in the brain and reproductive organs of females living with these males (Guzzo et al., 2013). Furthermore, pre-treating female mice with unlabelled E2 reduces the amount of radioactivity they display, which suggests that the labelled E2 remains bioactive in the receiving female and thus competes with the E2 already present in her system (Guzzo et al., 2013). Recent evidence shows, similar transfer of E2 from males to females’ reproductive tissues and brains in the big brown bat (deCatanzaro et al., 2014). Bats are more distant phylogenetically from humans and mice than the latter two species are from each other, suggesting that the capacity of E2 to transfer from males to females is ancient.  
 The VNO in mice may be involved in the transmission of sex steroids, though it does not appear to be necessary, and exposure to non-volatile sex steroids in urine likely contributes to both the Bruce and Vandenbergh effects. Ablation of the female’s vomeronasal organ (VNO) reduces the Bruce effect (Bellringer et al., 1980). However, as deCatanzaro (2015) asserts, female mice sniff the excretions of males, sucking its content into their system, and VNO ablation might simply block this sucking action. Male mice exposed to inseminated females or juvenile females show elevations in their creatinine-adjusted urinary E2 levels after a few days of exposure (Beaton et al., 2006; deCatanzaro et al., 2006, 2009), and these males also demonstrate polyuria, polydipsia, and active direction of their urine toward females (deCatanzaro et al., 2009). Unconjugated E2 and T are present in large quantities in the urine of male mice (deCatanzaro et al., 2006, 2009), and exposing female mice to just the urine of non-sire males is sufficient to cause pregnancy loss (Parkes and Bruce, 1962). Taken together, this evidence suggests that sex steroids are transmitted between conspecifics, at least in mice, but likely in mammals in general, and that this is not dependent on a VNO.  
  
1.4.3. Synthesis and Potential Pheromonal Transmission of Axillary Steroids in Humans  
 Steroids may be absorbed through human skin via direct contact between individuals, for example through perspiration transfer during intercourse. In fact, the engagement in sexual behaviour throughout the duration of the menstrual cycle, even when the female is not fertile, is an intriguing aspect of behaviour in humans (and possibly in a few other species) that could potentially play a role in pair bonding. However, it may also confer an adaptive advantage via communication through chemosignals. Regardless of the functionality (or lack thereof) of the human VNO, steroid hormones may still be transmitted between humans and may demonstrate pheromonal properties.   
 In humans, we suspect that transfer of perspiration, especially axillary perspiration, may allow transmission of sex steroids. There is evidence that axillary cells may excrete steroids originating from the blood (Brooksbank, 1970). However, there are peroxisomes located in the apocrine sweat glands of human axilla, suggesting that these cells can synthesize cholesterol, which is an essential precursor to steroid hormones including E2 (Rothardt and Beier, 2001). RT-PCR has revealed in these glands the presence of mRNAs of two peroxisome-associated enzymes used in the synthesis of cholesterol, namely mevalonate kinase and farnesyl diphosphate synthase (Rothardt and Beier, 2001). Apocrine sweat glands are primarily inactive until puberty, and they are prominent in the axilla, with some presence in the genital and mammary regions as well (Wilke et al., 2007). It has been suggested that women have more apocrine glands in their axilla than men do, and that men's apocrine glands are larger than women's (e.g. Hays, 2003). The apocrine glands' locations may allow for the transmission of apocrine secretions during intimacy, and their timing of development, as well as the potential sexual dimorphism they exhibit, implies that they may be relevant for reproduction. Apocrine sweating has been observed in response to painful and stressful stimuli (Shelley and Hurley, 1953), and it would be informative to investigate whether sexual arousal could also induce apocrine secretions. Wilke et al. (2007) suggest that since emotional (stressful) stimuli do not appear to cause axillary perspiration before puberty, the apocrine and apoeccrine glands, which appear to develop throughout puberty (Sato et al., 1987), may be largely responsible for emotional axillary perspiration. People vary in the number of apocrine glands they have, and levels of sex steroids observed in axillary perspiration could be affected by the number of apocrine glands as this could affect levels of available cholesterol.   
 Aromatase is the protein, encoded by the *CYP19* gene, that catalyzes the series of reactions which irreversibly convert C19 androgens to C18 estrogens; this enzyme is responsible for the conversion of T to E2 (see review by Simpson and Davis, 2001). Enzymes may inter-convert androgens, and possibly convert androgens to estrogens, in sebaceous glands, outer as well as inner root sheath cells of anagen terminal hair follicles, and dermal papilla cells, and all of these cells can be found in the axillary area (Fritsch et al., 2001; Sawaya and Price, 1997; Thornton et al., 2006). Furthermore, sebocytes (cells in the sebaceous glands), sweat glands, and possibly dermal papilla cells (all of which can be found in the underarm) appear to have the enzymes to convert certain precursors (dehydroepiandrosterone and androstenedione) into T and dihydrotestosterone, and T is itself a direct precursor to E2 (Zouboulis, 2007). Increasing activity of sebaceous glands is also seen before puberty, and is possibly mediated by adrenal androgens (Stewart et al., 1992). In sum, it appears that the skin of the axilla may be able to synthesize T and E2 de novo and/or from circulating precursors. However, more research is needed to understand the mechanisms by which steroid hormones are synthesized in the skin. In particular, biopsies of axillary skin to analyze aromatase expression and activity may be informative. Different alleles of the *CYP19* gene have already been associated with differences in breast cancer risk in women (Siegelmann-Danieli and Buetow, 1999), as well as differences in bone mineral density changes (Van Pottelbergh et al., 2003) and sperm concentration and motility in men (Lazaros et al., 2011). It is possible that these different alleles cause different degrees of E2 synthesis in the human underarm.   
 McGrath (2009) has suggested that enzymes in the axilla, including aromatase, may function to preserve androgen homeostasis, and that inhibiting apocrine sweat gland functioning through the use of antiperspirants may cause a build-up of the androgens made by these glands. If this is correct, it is possible that increased antiperspirant use could result in increased cutaneous synthesis of E2 and E1 by aromatase in order to compensate for excess androgens.   
 Other estrogen-and-androgen-like compounds that have been implicated as pheromones (such as the 16-androstene steroids) have also been detected in axillary perspiration. It is suspected that these compounds can be inter-converted by bacteria residing on the skin (e.g. Gower et al., 1994). Jackman and Noble (1983) observed substantial variation in human axillary bacterial species, with two common bacteria profiles being most frequently found, which were dominated either by coryneform or coccal flora.  
  
**1.5. Current Research: Purpose and Hypotheses**  
  
 The purpose of this research was to assess intra-individual stability in levels of E2 and T in adult males' axillary perspiration, which may ultimately lead to an understanding of the extent to which genetic vs. environmental factors contribute to the extraordinary variation observed in levels of these steroids. Participants, recruited from the David Braley Athletic Center at McMaster University, donated 4 perspiration samples with approximately 1-2 week intervals between samples. A questionnaire assessing environmental factors which may affect levels of sex steroids (e.g. dietary phytoestrogen consumption, stress level, and relationship status) was also administered. In addition, participants saw either a male researcher for the first 2 meetings and then a female researcher for the last 2 meetings, or vice versa, to assess whether the gender of the researcher would affect steroid levels. We suspect that genetic factors are partially responsible for determining the range within which adolescent males' axillary sex steroid levels will fall, but that environmental factors such as exposure to females also affect levels of axillary E2 and T. Accordingly, we expected to find modest intra-individual stability in axillary E2 and T. We also expected to find a wide inter-individual distribution of axillary E2 and T levels, as did Muir et al. (2008). If exposure to females does indeed affect axillary sex steroid levels, we expected that this exposure would be positively correlated with E2 and T, and that more prolonged exposure (e.g. via being in a committed romantic relationship with a woman) would be more strongly correlated with axillary E2 and T than would be shorter exposure (e.g. having a single recent sexual encounter with a female). We also suspected that phytoestrogen consumption could affect axillary E2 levels, and that it could be positively correlated, via cross-reaction with ELISA antibodies, or negatively correlated, via stimulating hypothalamic-pituitary feedback mechanisms. This study was exploratory with respect to the other environmental factors being assessed.   
 Another purpose of this research was to assess the value of a novel method of perspiration collection. Collecting droplets of pure perspiration can be difficult, as it requires participants to perspire to a great extent. Therefore cellulose filter paper swabs were used in addition to determine whether the steroids could be reliably extracted and measured. This could be a useful method in the future to collect perspiration from participants without necessitating strenuous exercise.

2.0. Materials and Methods  
  
**2.1. Participants**  
  
 The methodology was approved by the McMaster University Research Ethics Board (MREB). Participants were recruited via a recruitment poster (see Appendix A) and in person at the McMaster University David Braley Athletic Centre. Researchers invited young men at the running track, as well as “The Pulse” (a fitness and weight room), to learn about the study and to provide their email address if they were interested. Eighty-one males aged 20-30 years were recruited to give 4 perspiration samples with approximately 1-to 2-week intervals between samples. Of these, 73 participants gave at least one usable perspiration sample; the data from those who did not give at least one sample was excluded from analyses. Forty-nine of these 73 participants donated 4 usable (i.e. sufficiently large) perspiration samples. Participants were asked to complete a Physical Activity Readiness Questionnaire (see Appendix C) to ensure that they could safely exercise, and were not eligible for the study if they checked “yes” to any of the questions on this questionnaire. However, no participants answered “yes” to any of these questions. All participants read and signed a consent form (see Appendix B) and were assured of the anonymity of their data. Participants received a debriefing form after their first session (see Appendix E), and were offered $12 compensation for the first session, and $5 for each additional session, regardless of whether they were able to produce a usuable perspiration sample during the session.   
  **2.2. Assessment of Environmental Factors**  
  
 Each participant completed a paper and pencil questionnaire (see Appendix D) on the same day that the first perspiration sample was collected (Time 1). The questionnaire was designed to assess potential exclusion criteria as well as environmental factors which may contribute to E2 and/or T levels in axillary perspiration. Such factors included recent medications, stress level, diet, relationship status, sexual practices, and cohabitation (specifically whether the participant lives with any females). Exclusion criteria included whether subjects reported taking anabolic steroids or other medications that would clearly affect steroid hormone levels, but no participants reported taking such medications. The questionnaire was expected to take approximately 10 minutes to complete, but no time limit was set.   
 From the questionnaire responses, a composite score indicating general and sexual contact with females was created. This score consisted of four questions: whether the participant had sexual relations with any female in the past week; whether the participant was in a romantic relationship with a female; whether the participant, if in such a relationship, had regular sexual relations with his partner; and whether the participant, if in a relationship, lived with his partner. A score of 0 was applied where the answer was no, and a score of 1 was given where the answer was yes; the female-relationship composite was the sum of these scores, ranging from 0-4. A separate composite score indicating stress level was also created. Participants were asked to rate, on a scale of 0-5, how stressed they had been for the past month, and separately how stressed they had been over the most recent week, also on a scale of 0-5. The sum of these two stress-related responses was used to create the composite score.   
 Two researchers, one female and one male, conducted measures so that we could determine whether the gender of the researcher might affect axillary E2 and/or T levels. One researcher collected the first two perspiration samples, and the other collected the last two. Participants were counterbalanced with respect to the order in which they saw each researcher, with half seeing the female experimenter first and the others seeing the male experimenter first. Measures were conducted in public at the running track in the David Braley Athletic Centre.  
   
**2.3. Perspiration Collection**  
  
 Participants were asked not to wear deodorant or antiperspirant on the days that measures were to be conducted. All participants cleaned their underarms with hypoallergenic, unscented baby wipes, and then with alcohol swabs, in case any residue of antiperspirants or other toiletries were present. Participants were then asked to wear an unused polyethylene trash bag to promote perspiration. They were permitted to wear a shirt or sweater on top of the bag if they desired. Most participants chose to exercise for approximately 20-30 minutes before donating a perspiration sample, but exercise was not timed. After sufficient exercise had been performed, axillary perspiration was collected by gently scraping the underarm with a centrifuge tube. Perspiration was also collected on pre-weighed Whatman® 40mm grade 1 circular cellulose filter papers, by swabbing the underarm and then placing the filter paper into a pre-weighed centrifuge tube. Samples were placed in a cardboard box which was kept with ice packs inside a styrofoam case, before being transferred to a freezer. They were stored at ‑20ºC until assayed.  
  **2.4. Habitual Antiperspirant Use**  
  
 Subsequent to the data collection, we decided to inquire whether habitual antiperspirant use might affect steroid hormone levels in axillary perspiration, but no question about antiperspirant use was included in the original questionnaire. Therefore, an email was sent to each participant inviting them to answer a multiple choice question about their level of antiperspirant use (see Appendix E).   
 **2.5. Preparation of Filter Paper Samples**  
  
 Ethanol was used to extract steroids from 126 filter paper swabs. The pre-weighed centrifuge tubes (with the swabs inside) were post-weighed to calculate the weight of perspiration added to the swab. From this, the volume of perspiration was inferred by estimating that perspiration has a weight of 1 g/mL. One mL of ethanol was added to each centrifuge tube, which were then centrifuged at 1500 rpm for 15 minutes. The filter papers were removed from each tube using forceps, and the tubes were centrifuged again at 1500 rpm for 5 minutes. Tubes were then placed, open, under a fume hood for 300 hours to allow for complete evaporation of the liquid. Most samples evaporated completely in less than 150 hours, but great variation was observed in the evaporation time between samples. Of the 126 samples used, 2 did not evaporate completely in this time and were therefore discarded. To re-dissolve the steroids, 10μL of ethanol and 90μL of EIA phosphate buffer were added to each centrifuge tube, which were then spun at 1500 rpm for 5 minutes. These samples were then placed in the refrigerator at 4 °C. Before these samples were assayed, 100 additional μL of phosphate buffer was added, as preliminary experiments with different samples led us to suspect that the ethanol required further dilution so that it did not disrupt the pH of the assay. These samples were then assayed for E2 and T as described below.   
  
**2.6. Chemical Analysis**  
  
 Perspiration samples were analyzed using enzyme immunoassay procedures previously validated by Muir et al. (2008). Estradiol and testosterone standards were obtained from Sigma Chemical Co. (St. Louis. MO). Antibodies (anti-E2 R4972, anti-P CL425 and anti-T R156/7) and corresponding horseradish peroxidase conjugates were obtained from the Department of Population Health and Reproduction at the University of California, Davis. Perspiration samples were assayed in EIA phosphate buffer (0.1 mol/L sodium phosphate buffer, pH 7.0 containing 8.7 g of NaCl and 1 g of BSA per L) at 1:10 dilutions based on parallelism data. All samples were measurable in the sensitive range of the standard curve (approximately 50 % binding).   
 The samples which were prepared from the filter papers (as described above) were not diluted before being assayed. The samples of pure sweat were diluted as described below.

Assays for 17β-estradiol were carried out on NUNC® Maxisorb plates which were first coated with 50 μL of antibody stock (anti-E2 4972) diluted at 1:10000 in a coating buffer (50 mmolL bicarbonate buffer pH 9.6) and stored overnight at 4° C. Wash solution (0.15 mol/L NaCl solution containing 0.5 ml of Tween 20 per L) was added to each well to rinse away any unbound antibody, and then 50 μl EIA phosphate buffer (0.1 mol/L sodium phosphate buffer, pH 7.0 containing 8.7 g of NaCl and 1 g of BSA/L) per well was added. Twenty μL of standard or sample were added to each well immediately followed by 50 μL estradiol horseradish peroxidase diluted at 1:50,000 in EIA phosphate buffer and the plates were incubated for 2 hours at room temperature. Next, the plates were washed with wash solution and 100 μL of a substrate solution of citrate buffer and H2O2 , and 2,2'-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) diammonium salt (ABTS) was added to each well and the plates covered and incubated while shaking at room temperature for 30 – 60 minutes. The optical density of the plates was then read using a single filter at 405 nm using a microplate reader (Bio-Tek Instruments Inc. model number EL312E or ELx50). Blank absorbance was subtracted from each reading to account for nonspecific binding. Zero wells were considered to have 100 % enzyme conjugate binding to the antiserum and all standards and unknown values were divided by this mean zero reading. This gives a percent binding value. Crossreactivities for anti-estradiol antibody are: estradiol 100% , estrone 3.3% , progesterone 0.8% , testosterone 1.0% , androstenedione 1.0% , and all other steroids < 0.1%.  
 The testosterone assays were similar to the estradiol assay, except that the antibody stock (anti-T R156/7) was diluted at 1:10000 before plate coating and only 30 minutes elapsed between the first plate wash on the second day and the addition of the standards and samples. Finally, 50 μL of standard or sample was added to each well along with 50 μL of testosterone horseradish peroxidase diluted to 1:15000. Cross-reactivities for anti-testosterone antibody are: testosterone 100.0% , 5α-dihydrotestosterone 57.4% , androstenedione 0.27% , and androsterone, dehydroepiandrosterone (DHEA), cholesterol, estradiol, progesterone and pregnenolone < 0.05%.

**2.7. Statistical Analysis**

Data were analyzed using the programs in *Practical Statistics 4.0*, Canadian Academic Technology Inc., West Flamborough ON, copyright 1994. Pearson product-moment correlations were conducted among the measures, and their statistical significance was calculated by an associate t-test. Matched sample t-tests were applied to compare the average hormone measures obtained by the two experimenters. Multiple linear regression was applied to compare the questionnaire measures as predictors and the average of each hormonal measure. All statistical analyses were performed on data representing measurements from the pure vial samples (not the cellulose filter paper samples).

3.0. Results **3.1. Filter Paper Swab Method of Perspiration Collection**

Filter paper samples chosen at random (N=126) showed significant but small correlations with sex steroids in pure perspiration samples collected in vial tubes. E2 in the filter paper was positively correlated with E2 in the vial samples (r=+0.313, p=0.001), and T in the filter paper was positively correlated with T in the vial samples (r=+0.238, p=0.007). However, the mean E2 and mean T measured from the filter paper samples were both much smaller than the means measured from the vial samples. Furthermore, adequate parallelism was not observed in comparison of standard curves, meaning that the assay was not validated. Therefore, only vial samples, and not the cellulose filter paper swab samples, were used for statistical analysis.

**3.2. Questionnaire Responses**

All 81 participants filled out the questionnaire. However, not all were able to produce a viable perspiration sample. Thus the following questionnaire responses represent the 73 participants who were able to produce at least one perspiration sample.

Participants had a self-reported mean age of 22.9 years, most reported moderate stress levels (a mean of 2.6 on a 5-point scale), and 98.6% of participants reported exercising at least one hour per week (see **Table 1**). The majority of participants reported not consuming phytoestrogens (either soy, tofu, or flax seed) once per week or more on a regular basis (see **Table 1**).

The vast majority of participants (94.5%) reported preferring romantic relationships exclusively with women (vs. men), and 49.3% of participants were in a romantic relationship with a female at the time the questionnaire was administered, whereas no participants reported being in a romantic relationship with a male (see **Table 2**). Approximately one third of participants reported living with a female other than a romantic partner, and these females include family members and friends/roommates (see **Table 2**).

**Table 1:** Factors concerning age, stress level, diet and exercise habits of participants (N=73)

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Factor** | **Mean** | | **Standard Deviation** | | | | **Range** | | **No Answer** |
| **Age** | 22.90 | | 2.68 | | | | 20-30 | | 0 |
| **Standard drinks consumed in average week** | 3.35 | | 3.76 | | | | 0-15 | | 3 |
| **Standard drinks consumed last night** | 0.60 | | 1.46 | | | | 0-8 | | 0 |
| **Stress level in last month (on a 5-point scale)** | 2.62 | | 1.05 | | | | 0-4.5 | | 2 |
| **Stress level in last week (on a 5-point scale)** | 2.64 | | 1.22 | | | | 0-5 | | 0 |
| **Hours of exercise per week** | **0** | **1-3** | | **4-6** | | **7-9** | | **10+** | **No Answer** |
| 1 (1.4%) | 9 (12.3%) | | 24 (32.9%) | | 18 (24.7%) | | 21 (28.8%) | 0 (0%) |
| **Antiperspirant (Antp) Habit** | **No antp/ deodorant** | **Deodorant only** | | **Antp 1-2 days/week** | | **Antp 3-4 days/week** | | **Antp 5-7 days/week** | **No Answer** |
| 1 (1.4%) | 8 (10.9%) | | 2 (2.7%) | | 3 (4.1%) | | 20 (27.4%) | 39 (53.4%) |
|  | **Yes** | | | | **No** | | | | **No Answer** |
| **Soy consumption in last 24 hours** | 6 (8.2%) | | | | 66 (90.4%) | | | | 1 (1.4%) |
| **Flax consumption in last 24 hours** | 20 (27.4%) | | | | 51 (69.9%) | | | | 2 (2.7%) |
| **Soy consumption once per week or more** | 2 (2.7%) | | | | 71 (97.3%) | | | | 0 (0%) |
| **Tofu consumption once per week or more** | 1 (1.4%) | | | | 72 (98.6%) | | | | 0 (0%) |
| **Flax seed consumption once per week or more** | 18 (24.7%) | | | | 53 (72.6%) | | | | 2 (2.7%) |

**Table 2:** Factors concerning sexual preferences, relationship status, and habitation of participants (N=73)

|  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Factor** | **Exclusively Men** | **Mostly Men** | | **Men or Women** | | | **Mostly Women** | **Exclusively Women** | | **No Answer** |
| **Prefer to have romantic relationships with men, women, or both** | 1 (1.4%) | 0 (0%) | | 0 (0%) | | | 1 (1.4%) | 69 (94.5%) | | 2 (2.7%) |
|  | **Yes** | | | | **No** | | | | **No Answer** | |
| **Currently in a romantic relationship with a female** | 36 (49.3%) | | | | 37 (50.7%) | | | | 0 (0%) | |
| **If currently in romantic relationship with female, is partner on birth control pill** | 21 (28.8%) | | | | 14 (19.2%) | | | | 0 (0%) | |
|  | **Mean** | | **Standard Deviation** | | | **Range** | | | **No Answer** | |
| **If in relationship, duration of relationship (months)** | 28.88 | | 32.31 | | | 1.5-132 | | | 0 (0%) | |
| **If in relationship, number of weeks since partner last menstruated** | 1.93 | | 1.41 | | | 0-4 | | | 13 (17.8%) | |
| **If in relationship, frequency of sex (times/week)** | 1.95 | | 1.34 | | | 0-4.5 | | | 4 (5.5%) | |
|  | **Yes** | | | | **No** | | | | **No Answer** | |
| **Sex with any female in the last week, regardless of relationship status** | 28 (38.4%) | | | | 45 (61.6%) | | | | 0 (0%) | |
| **If sex with female in last week, is partner on birth control pill** | 13 (46.4%) | | | | 14 (50%) | | | | 1 (3.6%) | |
| **Currently in a romantic relationship with a male** | 0 (0%) | | | | 70 (95.9%) | | | | 3 (4.1%) | |
| **Sex with any male in the last week, regardless of relationship status** | 0 (0%) | | | | 70 (95.9%) | | | | 3 (4.1%) | |
| **Currently living with a romantic partner of any sex/gender** | 6 (8.2%) | | | | 67 (91.8%) | | | | 0 (0%) | |
| **Currently living with any other females besides a romantic partner** | 24 (32.9%) | | | | 49 (67.1%) | | | | 0 (0%) | |
| **If living with any other females, what is their relationship** | **Mother/Aunt/Sister/ Grandmother** | | | | **Roommate/Friend** | | | | **No Answer** | |
| 8 (33.3%) | | | | 16 (66.7%) | | | | 0 (0%) | |
| **If living with romantic partner, is partner male or female** | **Male** | | | | **Female** | | | | **No Answer** | |
| 0 (0%) | | | | 6 (100%) | | | | 0 (0%) | |
| **If partner with whom participant is living is female, does she take the birth control pill** | **Yes** | | | | **No** | | | | **No Answer** | |
| 1 (16.7%) | | | | 5 (83.3%) | | | | 0 (0%) | |

**3.3. Estradiol Highlights**

Axillary E2 levels ranged from approximately 1 to 710 ng/mL (see **Figure 1**) with a mean of 90 ng/mL and standard error of 20 (see **Table 3**). Axillary E2 levels were, in general, fairly stable within an individual. Among those who donated 4 perspiration samples, E2 at Time 1 correlated very strongly with average E2 (r=+0.64, t(47)=5.76, p<0.0001; see **Table 4**). Interestingly, E2 levels were more stable within the female experimenter’s measures (r=+0.83, t(47)=10.23, p<0.0001) than the male experimenter’s measures (r=+0.20, t(47)=1.40, p=.17), i.e. E2 levels from the two sessions conducted by the female experimenter correlated more strongly with one another than levels from the two sessions conducted by the male experimenter. Within an individual, the average E2 as collected by the female experimenter correlated modestly with the average E2 as collected by the male experimenter (r=+0.31, t(57)=2.43, p=0.02; see **Table 6**). A very small effect of experimenter was observed (with E2 levels trending towards being higher with the female experimenter), but this was not statistically significant among participants who donated all 4 perspiration samples (two-tailed p=0.18) or among participants who donated at least one sample to each experimenter (p= 0.21). No correlation with age, and no effect of testing number, were observed. No significant correlation with self-reported stress level was observed. Among participants who gave at least one sample to each experimenter, a composite score indicating recent sexual/romantic contact with women correlated positively with average E2 levels (r=+0.29, t(57)=2.26, p=0.03; see **Table 6**). This relationship appears to be driven by the E2 measures conducted by the female researcher (r=+0.31, t(57)= 2.44, p=0.02), however, because the E2 as measured by the male researcher was not significantly correlated with the composite score (r=+0.07, t(57)= 0.52, p=0.61; see **Table 6**).

**Figure 1**: Distribution of axillary E2 levels (N=73), using the average value of the samples for each participant. The mean (of the within-subject averages) is 90 ng/mL. Labels on the x-axis represent midpoints of bins, each of which represents a range of 10 ng/mL.



**3.4. Testosterone Highlights**  
 Axillary T levels ranged from 3 to 645 ng/mL (see **Figure 2**), with a mean of 195 ng/mL and standard error of 22 (see **Table 3**). Overall, axillary T levels correlated strongly with axillary E2 levels, and showed similar intra-individual stability. Among those who donated all four perspiration samples, average T correlated significantly with average E2 (r=+0.61, t(47)= 5.33, p<0.0001; see **Table 4**). Axillary E2 during each meeting (Time 1, Time 2, Time 3, and Time 4) correlated significantly with axillary T from the same meeting (all p<0.0001; see **Table 4**). Average T levels as collected by the male researcher correlated significantly with average T levels as collected by the female researcher (r=+0.77, t(47)= 8.33, p<0.0001; see **Table 4**). No correlation with age, and no effect of testing number, were observed. No significant correlation with self-reported stress level was observed. Among participants who gave at least one sample to each experimenter, the composite score indicating recent sexual/romantic contact with women correlated positively (as a trend but not statistically significantly) with average T levels among measures conducted by the female researcher (r=+0.24, t(57)=1.85, p=0.07; see **Table 6**), but not among measures conducted by the male researcher (r=-0.05, t(57)= 0.39, p=0.70; see **Table 6**).

**3.5. Correlations among Steroid Measures on Perspiration Samples from Subjects Giving Four Perspiration Samples**

Among the participants who gave all four perspiration samples (N=49), nearly all correlations among steroid measures were significant (see **Table 4**). However, the correlation between E2 levels at session 2 and E2 levels at session 3 (i.e. the 2 sessions between which the participants switched from the male to female experimenter or vice versa) was very low (r=+0.16, t(47)=1.09, p=0.28).  
  
**3.6. Analysis of Experimenter Effects on Axillary E2 and T**

Among participants who gave all four perspiration samples, axillary E2 levels were slightly higher when collected by the female experimenter, but this was not statistically significant (t(48)=1.35, p=0.18). Among participants who gave all four perspiration samples, axillary T levels were significantly higher when collected by the female experimenter (t(48)=3.53, p=0.001). The same results were seen when all participants who donated at least one sample were considered (for E2: t(58)=1.26, p=0.21; and for T: t(58)=2.57, p=0.01).

**Figure 2**: Distribution of axillary T levels (N=73), using the average value of the samples for each participant. The mean (of the within-subject averages) is 195 ng/mL. Labels on the x-axis represent midpoints of bins, each of which represents a range of 10 ng/mL.



**Table 3:** Descriptive statistics for axillary E2 and T, as collected by either a female or male researcher

|  |  |
| --- | --- |
| **Variable** | **Mean (ng/mL) ± Standard Error** |
| **E2 at session 1** | 75.84 ± 19.84 |
| **E2 at session 2** | 68.20 ± 13.72 |
| **E2 at session 3** | 80.47 ± 24.56 |
| **E2 at session 4** | 135.52 ± 43.31 |
| **Average E2** | 90.01 ± 19.85 |
| **Average E2 as collected by female experimenter** | 110.00 ± 31.09 |
| **Average E2 as collected by male experimenter** | 70.015 ± 16.07 |
| **T at session 1** | 186.85 ± 24.17 |
| **T at session 3** | 209.94 ± 29.07 |
| **T at session 3** | 154.64 ± 21.04 |
| **T at session 4** | 228.63 ± 29.86 |
| **Average T** | 195.02 ± 22.13 |
| **Average T as collected by female experimenter** | 225.33 ± 27.00 |
| **Average T as collected by male experimenter** | 164.70 ± 19.94 |

**Table 4:** Pearson correlations among measures of testosterone (T) and estradiol (E2) in axillary perspiration of men, as collected by either a male or female experimenter

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | **Average T as collected by male** | **Average T as collected by female** | **Average T** | **T at session 4** | **T at session 3** | **T at session 2** | **T at session 1** | **Average E2 as collected by male** | **Average E2 as collected by female** | **Average E2** | **E2 at session 4** | **E2 at session 3** | **E2 at session 2** |
| **E2 at session 1** | +.393\* | +.506\* | +.486\* | +.455\* | +.393\* | +.353\* | +.450\* | +.774\* | +.422\* | +.644\* | +.263 | +.568\* | +.430\* |
| **E2 at session 2** | +.406\* | +.497\* | +.487\* | +.285\* | +.187\* | +.706\* | +.418\* | +.597\* | +.276 | +.458\* | +.237 | +.157 |  |
| **E2 at session 3** | +.350\* | +.462\* | +.440\* | +.504\* | +.620\* | +.139\* | +.281\* | +.457\* | +.904\* | +.893\* | +.760\* |  |  |
| **E2 at session 4** | +.435\* | +.496\* | +.499\* | +.585\* | +.519\* | +.279\* | +.316\* | +.444\* | +.903\* | +.887\* |  |  |  |
| **Average E2** | +.514\* | +.626\* | +.613\* | +.638\* | +.605\* | +.405\* | +.444\* | +.680\* | +.925\* |  |  |  |  |
| **Average E2 as collected by female** | +.351\* | +.563\* | +.502\* | +.534\* | +.535\* | +.301\* | +.350\* | +.352\* |  |  |  |  |  |
| **Average E2 as collected by male** | +.590\* | +.457\* | +.545\* | +.543\* | +.460\* | +.419\* | +.420\* |  |  |  |  |  |  |
| **T at session 1** | +.761\* | +.847\* | +.860\* | +.724\* | +.569\* | +.631\* |  |  |  |  |  |  |  |
| **T at session 2** | +.665\* | +.824\* | +.802\* | +.591\* | +.429\* |  |  |  |  |  |  |  |  |
| **T at session 3** | +.820\* | +.713\* | +.805\* | +.802\* |  |  |  |  |  |  |  |  |  |
| **T at session 4** | +.891\* | +.850\* | +.920\* |  |  |  |  |  |  |  |  |  |  |
| **Average T** | +.922\* | +.958\* |  |  |  |  |  |  |  |  |  |  |  |
| **Average T as collected by female** | +.772\* |  |  |  |  |  |  |  |  |  |  |  |  |

\* = Bivariate 2-tailed p < 0.05.

**3.7. Analysis of Questionnaire Data**

Several composite scores (see **Table 5**) were made and included in multiple regression analyses of the questionnaire data. The ‘partner on birth control’ composite was made by summing the responses to questions 13a, 15c, and 17b (see Appendix C), where ‘no’ or no answer=0 and ‘yes’=1. The ‘relations with women’ composite consisted of four questions: whether the participant had sexual relations with any female in the past week; whether the participant was in a romantic relationship with a female; whether the participant, if in such a relationship, had regular sexual relations with his partner; and whether the participant, if in a relationship, lived with his partner. A score of 0 was applied where the answer was no, and a score of 1 was given where the answer was yes; the female-relationship composite was the sum of these scores, ranging from 0-4. The ‘homosexuality’ score was the result of question 12 (see Appendix C), where ‘exclusively men’=4, ‘mostly men’=3, ‘men or women’=2, ‘mostly women’=1, and ‘exclusively women’ or no answer=0. The ‘stress’ composite was made by summing the responses of questions 9 and 10 (the two stress-related questions on the questionnaire; see Appendix C). The ‘phytoestrogen’ composite was made by summing the responses of questions 4-8 (the five phytoestrogen-related questions on the questionnaire; see Appendix C), where ‘no’ or no answer=0 and ‘yes’=1. The exercise score was made by taking the midpoint of the participant’s response, or giving a score of 0 if ‘0’ was selected, or 10 if ‘10+’ was selected.

A multiple regression analysis using age, the phytoestrogen composite, stress composite, exercise score, homosexuality score, relations with females composite, and birth control composite as predictors did not significantly explain the variance found in axillary E2 (R2=0.22, F(7,51)=2.01, p=0.07). However, this was significant among the measures conducted by the female researcher (R2=0.23, F(7,51)=2.21, p=0.05). Among measures conducted by the male researcher, the model predicted a much smaller amount of the variation in E2 (R2=0.08, F(7,51)=0.66, p=0.71). A similar pattern was seen when the same model was applied to axillary T levels. The model did significantly explain the variance in T among all of the sessions (R2=0.25, F(7,51)=2.46, p=0.03), but when only the measures conducted by the female researcher were considered, a much greater proportion of the variance was explained (R2=0.29, F(7,51)=3.02, p=0.01) than when the model was applied to the measures conducted by the male researcher (R2=0.17, F(7,51)=1.44, p=0.21).

**3.8. Habitual Antiperspirant Use**

Antiperspirant use (summarized in **Table 1**) was not significantly correlated with either average E2 (r=+0.02, p=0.92), or average T (r=-0.23, p=0.20). For these data, average E2 and average T were calculated as the mean of the values for that participant (such that if the participant had only donated one perspiration sample, that one value would be considered their mean value).

**Table 5:** Descriptive statistics for composite scores from questionnaire responses (N=59)

|  |  |
| --- | --- |
| **Variable** | **Mean ± SE** |
| **Phytoestrogen composite score** | 0.58 ± 0.12 |
| **Stress composite score** | 5.29 ± 0.26 |
| **Exercise score** | 6.68 ± 0.37 |
| **Homosexuality score** | 0.10 ± 0.09 |
| **Relations with Women Composite Score** | 1.29 ± 0.19 |
| **Partner on birth control composite score** | 0.46 ± 0.09 |

**Table 6:** Pearson correlations among measures of testosterone (T), estradiol (E2), and questionnaire responses, as collected by either a male or female experimenter (N=59)

|  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | **Partner on birth control composite score** | **Relations with women composite score** | **Homo- sexuality score** | **Exercise score** | **Stress composite score** | **Phyto- estrogen composite score** | **Age** | **Average T as collected by male** | **Average T as collected by female** | **Average T** | **Average E2 as collected by male** | **Average E2 as collected by female** |
| **Average E2** | -.051 | +.287\* | -.902 | +.038 | -.170 | -.080 | +.069 | +.478\* | +.595\* | +.602\* | +.650\* | +.919\* |
| **Average E2 as collected by female** | -.093 | +.307\* | -.070 | +.023 | -.095 | -.088 | +.084 | +.288\* | +.555\* | +.491\* | +.306\* |  |
| **Average E2 as collected by male** | +.055 | +.068 | -.088 | +.077 | -.231 | -.027 | -.029 | +.635\* | +.341\* | +.492\* |  |  |
| **Average T** | -.199 | +.146 | -.184 | -.316\* | -.198 | -.154 | +.172 | +.850\* | +.944\* |  |  |  |
| **Average T as collected by female** | -.199 | +.238 | -.181 | -.359\* | -.137 | -.118 | +.229 | +.640\* |  |  |  |  |
| **Average T as collected by male** | -.143 | -.052 | -.149 | -.165 | -.244 | -.171 | +.005 |  |  |  |  |  |
| **Age** | -.150 | +.341\* | -.073 | -.431\* | -.018 | -.025 |  |  |  |  |  |  |
| **Phytoestrogen composite score** | +.122 | -.038 | -.101 | +.034 | -.203 |  |  |  |  |  |  |  |
| **Stress composite score** | -.097 | +.007 | +.175 | +.143 |  |  |  |  |  |  |  |  |
| **Hours of exercise per week** | +.162 | -.152 | +.045 |  |  |  |  |  |  |  |  |  |
| **Homosexuality score** | -.102 | -.141 |  |  |  |  |  |  |  |  |  |  |
| **Relations with Women Composite Score** | +.485\* |  |  |  |  |  |  |  |  |  |  |  |

\* = Bivariate 2-tailed p < 0.05.

4.0. Discussion  **4.1. Summary and General Interpretation of Findings** This research was undertaken to investigate intra-individual stability in levels of axillary E2 and T in men, as well as correlations of these hormones with various environmental factors. Axillary E2 levels demonstrated a wide inter-individual range but were, in general, fairly stable within an individual. E2 at Time 1 correlated very strongly with average E2, suggesting that one measure of an individual's axillary E2 is a fairly reliable indicator of their average levels. However, E2 levels were more stable within the female experimenter’s measures than the male experimenter’s measures, i.e. E2 levels in perspiration collected by the female experimenter correlated more strongly with one another than levels in perspiration collected by the male experimenter. Within an individual, the average E2 as measured by the female experimenter correlated modestly with the average E2 as measured by the male experimenter, but axillary E2 levels were slightly higher when measured by the female experimenter (though this trend was not statistically significant). Among the participants who gave all four perspiration samples, nearly all correlations among steroid measures were significant. However, the correlation between E2 levels at session 2 and E2 levels at session 3 (i.e. the 2 sessions between which the participants switched from the male to female experimenter or vice versa) was very low.  
 Axillary T levels also demonstrated a wide inter-individual range but were also fairly stable within an individual, and axillary T levels correlated strongly with axillary E2 levels. Positive correlations were observed between average T levels as measured by the male researcher and average T levels as measured by the female researcher. However, axillary T levels were significantly higher when measured by the female experimenter.  
 Muir et al. (2008) found that men’s axillary E2 ranged among individuals from 2 to 397 ng/ml, and axillary T ranged from 18 to 1671 ng/ml. In this study we found that axillary E2 ranged from 1 to 710 ng/mL demonstrating an even wider range than was previously observed by Muir et al. (2008), whereas axillary T ranged from 3 to 645 ng/mL, demonstrating a narrower range.   
 The findings in this project suggest that, while levels of axillary E2 and T in men tend to be fairly stable, they do vary to some extent, possibly due to environmental triggers. These findings are consistent with our suspicion that genetic factors are partly responsible for levels of axillary E2 and T in men, and suggest that contact with conspecifics can also affect these hormones. Specifically, recent contact with a female may stimulate an increase in levels of axillary E2 and T in men (or at least in heterosexual men—it should be noted that since only one participant reported dating men, trends could not be compared for participants who date men vs. participants who date women).   
 Interestingly, the regression predicting E2 from the questionnaire data were only significant for the measures conducted by the female experimenter, and a similar pattern was seen with T. Additionally, the greatest predictor (and simple correlate in the correlation matrix) was having more relations with females. The data suggest that having more interactions with females increases both E2 and T in axillary perspiration of men, and that the immediate measure may be sensitized by the presence of a woman during sampling. One caveat is that this is a correlational relationship that does not prove causality. It is possible that men with higher pre-existing E2 and T are more likely to attract women. In addition, these data were collected using only one male and one female experimenter, and it is therefore not clear whether any effects seen were due to the gender of the experimenter or to some other trait. Therefore, it would be useful to conduct a similar study using several female and several male researchers. Overall, when considered in the context of the animal literature—which strongly suggests that E2 acts as a pheromone in mice and other mammals (see review by deCatanzaro, 2015)—this study’s findings are consistent with our hypothesis that E2 may have pheromonal properties in humans. T is also a potential candidate for a human pheromone, and it has similar chemical properties to E2 which would allow it to be transferred between individuals, though in mice T administered to the nasal area does not transfer into the body quite as readily as does E2 (Guzzo et al., 2012).  
 Since the gym is a public space, participants may have come into contact with female gym members during the study, which may have diminished the effect of the researcher's gender by augmenting axillary E2 and T levels during the measures conducted by the male researcher. It is possible that contact with females outside of the gym also occurred (e.g. some participants may have spent time with a girlfriend prior to some of the sessions). In general, the participants who met with the male researcher may or may not have had any contact with women on the days of their meetings, whereas the participants who met with the female researcher did have contact with at least one woman. If recent contact with women does increase axillary E2 and T in men (or at least, in heterosexual men), this may explain the higher intra-individual stability found in the measures conducted by the female researcher.   
 It is possible that factors which were not assessed affect axillary E2 and T in men. For example, environmental factors which remained stable throughout the duration of the experiment but which were not assessed by the questionnaire may have contributed to the intra-individual stability observed. Developmental factors, ranging from the prenatal period to the post-pubertal period, may also influence this variation. These factors may include nutrition, various stressors, and/or contact with other humans prior to the commencement of the study.   
  
**4.2. Effectiveness of Filter Paper Swab Method of Perspiration Collection**  
 The cellulose filter paper perspiration collection method was not proven to provide a reliable measure of E2 or T. However, if this method can be modified to create a reliable method for future studies, it could provide a useful way to collect perspiration samples from participants without necessitating excessive perspiration. One noteworthy aspect of this filter paper swab method is the dramatic difference in evaporation time of the ethanol among samples, after the steroids were extracted from the filter papers. Most samples had completely evaporated after fewer than 150 hours, but a very small subset took over 300 hours to evaporate. This is possibly due to different levels of salts in different samples. Furthermore, it is possible that some samples contained small amounts of sebum which never evaporated, and that the few samples which did not evaporate completely had high sebum content and therefore left visible droplets of sebum behind. Nearly every sample had miniscule amounts of visible residue at the bottom of the centrifuge tube (see Appendix G), which possibly contained hormones, salts, and sebum, but this would likely cause a negligible difference in the volumes of the samples after the ethanol and phosphate buffered saline were added, except in the cases of the few samples with visible drops of liquid that did not evaporate.   
 We suspect that the ethanol used to re-dissolve the steroids may have affected the performance of the ELISAs. Ethanol may have altered the pH of the solutions, affecting the ability of the antibody to bind to the wells.

**4.3. Conspecific-Mediated Endocrine Changes in Rodents**

In rodents (and possibly in other mammals), there is evidence that E2, and possibly T, have pheromonal properties, and that these sex steroids are transmitted from males to females via urine. Plasma E2 increases dramatically in female mice after male exposure (Bronson and Desjardins, 1974), and increasing evidence implicates E2 transfer in both the Bruce and Vandenbergh effects (deCatanzaro, 2015).

Proximity to female mice causes males to urinate more, progressively over days of exposure, and males tend to direct urine towards females' cage compartments (deCatanzaro et al., 2009). Female-exposed males have more dilute urine, as urinary creatinine of isolated males exceeds that of female-exposed males, and female-exposed males have been found to drink more water. However, creatinine-adjusted E2 and T of female-exposed males exceeds that of isolated males. These findings suggest that E2 and T might increase in male mice exposed to females, and that males may use urine as a means of transferring these steroids to proximate females. This behaviour could be adaptive, as the transfer of sex steroids may contribute to the hastening of reproductive maturation of proximate females, the termination of early pregnancies sired by other males, as well as female sexual receptivity and ovulation. It is likely not common for urine transfer to occur between humans, but it is possible that these sex steroids could transfer between humans via other routes. Namely, perspiration could provide a means of steroid transfer between humans who have direct contact (e.g. through sexual relations).

Endocrine changes appear to be affected by whether conspecifics are familiar or novel. After 1 week, male house mice paired with familiar females do not have higher plasma T levels than do males that remain in all-male groups, but paired males show increased T 30 to 60 minutes after the resident female is replaced by another female (Macrides et al., 1975). Batty (1978) has also reported that close proximity of estrous female mice increases plasma T levels in some strains of mice within 15 minutes. Immediately after a sexual behaviour test in these mice, plasma T was higher in males showing sexual responses (Batty, 1978). There were correlations between T levels and mount latency, and T was greatest at the initiation of mounting responses, and declined during copulation, although not significantly. These findings suggest that the possibility of mating with a novel female could induce endocrine changes in male rodents (and/or that the endocrine changes could promote sexual interest in the male), but that familiar females might not induce such changes. It has also been suggested by Bliss et al. (1972) that both acute and chronic stress, as well as sexual satiety, reduce testicular function in rodents, whereas sexual excitation increases it.

Overall, in rodents, encounters with the opposite sex have been noted to produce endocrine changes in members of both sexes. Due to the highly conserved nature of steroid dynamics in mammals, it is possible that similar mechanisms exist in humans. Indeed, in men, plasma T has been noted to be higher during periods of sexual activity, but men who are more sexually active and experience orgasm more frequently have lower mean plasma T (Kraemer et al., 1976). More research is needed to understand the dynamics of axillary E2 and T in response to conspecifics in humans. Future studies could aim to investigate how these hormone levels change in response to novel and familiar individuals of the opposite sex. Individuals who do not identify as heterosexual could also be investigated, e.g. to assess whether levels of axillary E2 and T in homosexual men change in response to novel men. Future studies could also investigate both heterosexual and homosexual women to assess whether their levels of axillary E2 and T change in response to conspecifics. It may also be worth investigating whether E2 and T levels change in the serum and in other sources of perspiration (e.g. facial perspiration) in response to conspecifics; however, previous evidence has already found that levels of these sex steroids in the axilla do not tend to correlate with serum or other perspiration samples from the same individual (Muir et al., 2008).  **4.4. Potential Adaptive Significance of Axillary Sex Steroids and Their Transmission in Humans**  
  
 Certain men produce very high quantities of axillary sex steroids, specifically T and E2, and genetic factors may contribute to this. If sex steroids are transferred between humans, then females with whom these men have regular direct contact could potentially have more regular menstrual cycles, more frequent ovulation, and increased sexual desire. If this trait of producing high levels of axillary steroids is indeed heritable, and if it could potentially lead to higher fitness via these mechanisms, then it can be argued to be adaptive. More evidence is needed before it can be concluded that higher axillary sex steroid content could lead to higher fitness, but it is certainly plausible. For example, there is some evidence that exogenous steroid hormones affect mood and sexual desire in women. Bensafi et al. (2004) examined the effects of sniffing 4,16-androstadien-3-one (AND), an androgen, and 1,3,5(10),16-estratetraen-3-ol (EST), an estrogen (more volatile and much less potent than E2), on mood, memory, and autonomic nervous system (ANS) responses. They found that the effects of these compounds were contextually dependent on mood. They induced neutral, sad, happy, and sexually aroused moods using film clips. During the neutral mood, none of the compounds affected mood or ANS function, but both compounds increased sexual arousal during the aroused mood, and AND had various effects on mood and skin temperature. It would be worth investigating whether other estrogens and/or androgens can have similar effects on arousal and mood. It may be informative to investigate whether E2 and/or T can affect sexual arousal in doses comparable to levels seen in males' axillary perspiration, in the contexts of various moods, because the potential ability to increase arousal in already aroused females could be one advantage in males who produce high levels of these steroids.  
 If E2 and T have pheromonal properties in humans, they may affect mate choice, although it is currently unclear whether they can do this. For example, Rantala et al. (2006) found that neither T nor E2 levels in men correlated significantly with females' ratings of male attractiveness as gauged by perspiration scent. However, this study measured salivary steroids, and sex steroid content in saliva generally does not correlate with sex steroid content in axillary perspiration (Muir et al., 2008). Furthermore, E2 and T are not odorous, and would exert their effects on physiology and behaviour through absorption and binding to receptors, not through odour.   
 Sex steroids are not volatile, so direct contact is required for their transmission. This transmission may therefore be much more likely in couples who have already begun a sexual relationship. If higher axillary E2 and/or T does confer an adaptive advantage via pheromonal activity, it is possible that this advantage does not come from an effect on desirability as a mate, but rather an effect on fertility in a female with whom the male has already mated. It may therefore be informative to investigate axillary E2 and T levels in bonded couples and to examine whether they correlate with various aspects of fertility. Indeed, relationship status has already been shown to affect steroid dynamics to some extent; men who are in committed, romantic relationships have lower salivary T than single men (Burnham et al., 2003). If E2 and T do indeed have pheromonal properties in humans, they may affect, or be affected by, sexual practices, fertility, and reproduction in bonded couples. For example, T may increase sexual desire in women. There is some evidence that transdermal T can increase libido in postmenopausal women with decreased sexual interest, despite serum levels of T appearing not to correlate with sexual desire or function (see review by Basson, 2010). A recent review by Davis et al. (2012) concluded that the transdermal testosterone patch is effective for treating low libido in menopausal women with and without concomitant estrogen or estrogen/progestin therapy. It has not been investigated whether T would increase sexual desire in healthy women who do not have a sex disorder, but this would be difficult to elucidate given the ethical issues associated with administering sex steroids to individuals who do not demonstrate a need for them. It would be worth investigating average sex steroid levels in bonded couples, as well as hormone levels before, during, and/or after intercourse. If our novel method of perspiration collection using cellulose filter paper swabs can be improved, it might provide a more convenient method of sampling which could facilitate such studies.  
 If the Vandenbergh effect does exist in humans, then this could potentially increase a male's fitness by increasing the fertility of a proximal female. However, it is currently unclear whether the presence of unrelated males will hasten the age of reproductive maturation of prepubertal girls. One study—which used self-report measures and relied on participants' memory of when they began puberty and with whom they were residing during their adolescence—has reported that father absence predicts an earlier age of puberty in girls, but that stepfather presence is not a predictor (Bogaert, 2005). Another study assessed participants in the sixth grade and then followed up one year later, and used established scales to assess pubertal development; this study reported that stepfather presence was an even stronger predictor of early menarche than father absence (Ellis and Garber, 2000). In addition, recent data indicates a possible Vandenbergh effect in the wild gelada, a primate (Lu and Beehner, 2012). This suggests that a mechanism analogous to the Vandenbergh effect may exist in humans and that further studies are warranted.   
 If the Bruce effect exists in humans, this may also have adaptive significance, as it may help to prevent males from raising offspring that are not their own. It may also confer an advantage for a female, as women invest a lot of time and energy in offspring. Therefore, if there are desirable mates nearby, with whom a female might be able to produce high quality offspring, the female may be wish to abort a current early pregnancy with a less desirable partner. In species whose members may perform infanticide, the Bruce effect may confer higher fitness for a female’s offspring by making them less likely to be killed by a novel male. It is currently unclear whether a Bruce effect exists in humans, but it has been described in a number of mammals including the wild gelada (Roberts et al., 2012). Given the highly conserved nature of steroid dynamics, it may be worth investigating whether a mechanism analogous to the Bruce effect could exist in humans.  
 Overall, increasing evidence clearly implicates E2 as a pheromone in rodents and other mammals, and given T’s similar potential for transdermal absorption, as well as the known effects that it has on reproductive physiology and behaviour, a case can be made for T being a potential pheromone as well. Thus it is worth investigating further the potential pheromonal properties and effects on fitness that these hormones may demonstrate in humans.  **4.5. Limitations of Current Research** Participants perspired to different degrees during their sessions, firstly because levels of hydration and water consumption were not controlled, and secondly because exercise was not timed. Since neither E2 nor T are volatile compounds, it is possible that quantities remained behind on the participants’ skin while the water in their perspiration evaporated. If this happened differentially among individuals, or within individuals across sessions, this may have introduced bias.

Furthermore, each session was conducted in public, where the participant was exposed to both men and women, which may have altered the effect of the researcher on the participants’ axillary sex steroid levels. In addition, seasonal and temporal changes have been shown to affect serum levels of T (Barberia et al., 1973; Dabbs, 1990), and it is possible that these factors affect E2 as well. In this study, all sessions were conducted in the late winter/early spring between 11 am and 4 pm.

Lastly, crossreactivities of the ELISA are a consideration here; however, the purpose of this work was not to assess the absolute value of axillary sex steroid concentration but the variability.  
 **4.6. Other Future Directions**  
 To acquire further evidence about the potential genetic factors responsible for variation in axillary sex steroids, investigating familial concordance, for example using pairs of brothers, would be useful. In particular, if monozygotic twin pairs show more similarity in these traits than do dizygotic twin pairs, this would be good evidence that genetic factors affect levels of axillary E2 and T. To further investigate the possibility of contact with novel females affecting axillary sex steroids, it would be informative to conduct a study similar to the present one, using several male and several female researchers, and to expose each participant to only members of one gender during each perspiration donation.   
 Many practical and ethical limitations exist with respect to the investigation of potential pheromones in humans. For example, it would be informative to inject human participants with radiolabelled E2 and T, and to assess whether these hormones transfer to conspecifics, but this is not a realistic endeavour for ethical reasons. However, useful knowledge could still be gained from research regarding the physiology of sex steroid production in the axillary region. To do this, skin biopsies could be used to analyze aromatase activity in the underarm. It is possible that variation in the *CYP19* gene may cause variation in aromatase activity in the axilla, and that this may contribute to different levels of axillary T and/or E2. Another interesting question is whether exposure to certain endocrine disrupting chemicals (EDCs) such as bisphenol-A (BPA) may affect axillary sex steroid levels in humans, possibly by affecting *CYP19* gene expression; indeed, EDCs have been shown to affect the expression of this gene in teleost fish (Cheshenko et al., 2008).

In addition to this work's implications regarding potential pheromonal communication between humans, hormone levels in human axillary perspiration may have clinical implications as well. It has been hypothesized that hormone-dependent cancers may be more likely to arise in those with increased xenoestrogen exposure and/or increased transdermal absorption of their own sex steroids caused by aluminum-based antiperspirants, and global sales of antiperspirants have paralleled trends in the incidence and mortality from breast and prostate cancer (see review by McGrath, 2009). A link between hormone-dependent cancers and antiperspirants has not yet been proven, but it may still be worth investigating potential relationships between axillary sex steroids, aluminum-based cosmetic products, and hormone-dependent cancers.  
 Overall, if E2 and/or T are transmitted between humans, they may affect mate choice, as well as potentially promoting sexual receptivity, fertility, and/or the regulation of the menstrual cycle. Given the extraordinary levels of E2 and T in the axillary perspiration of some males, the chemical properties of these compounds which would allow transdermal absorption, and the potential physiological effects of exogenous E2 and T, it is possible that these hormones have pheromonal properties in humans.

**4.7. Conclusion**

This research found wide inter-individual ranges and notable intra-individual stability in levels of axillary E2 and T in men. Axillary E2 and T were both higher when the perspiration was collected by a female researcher as opposed to a male researcher, though the levels of E2 were not significantly higher. In addition, a composite score indicating recent sexual/romantic contact with women correlated positively with average E2 levels, and this relationship was much stronger among the E2 measures conducted by the female researcher than the male researcher. A multiple regression analysis using age, the phytoestrogen composite, stress composite, exercise score, homosexuality composite, relations with females composite, and birth control composite as predictors did not significantly explain the variance found in axillary E2 when all data points were taken into consideration. However, the model did significantly predict E2 levels among the measures conducted by the female researcher. When the same model was applied to axillary T levels, it did significantly explain the variance in T among all of the sessions, but when only the measures conducted by the female researcher were considered, a much greater proportion of the variance was explained than when the model was applied to the measures conducted by the male researcher. The cellulose filter paper perspiration collection method was not proven to provide a reliable measure of E2 or T; however, it may be possible to modify and improve this method for future studies.

These findings are consistent with our suspicion that genetic factors, and/or possibly inter-individual axillary steroid differences related to unknown developmental factors, are partly responsible for levels of axillary E2 and T in men. The findings also suggest that contact with conspecifics affects levels of these hormones. More research is needed to fully understand the dynamics of axillary E2 and T in response to conspecifics in humans.

5.0. References   
  
Alonso, L.C., Rosenfield, R.L., 2002. Oestrogens and puberty. Best Pract. Res. Clin. Endocrinol. Metab. 16, 13-30.   
  
Anderson, J.N., Peck, E.J., Clark, S., 1975. Estrogen-induced uterine responses and growth: relationship to receptor estrogen binding by uterine nuclei1 1. Endocrinology 96(1), 160-167.  
  
Apter, D., Vihko, R., 1985. Premenarcheal endocrine changes in relation to age at menarche. Clin. Endocrinol. 22, 753-760.  
  
Barth, J.H., Kealey, T., 1991. Androgen metabolism by isolated human axillary apocrine glands in *Hidradenitis suppurativa*. Br. J. Dermatol. 125, 304-308.  
  
Barton, D. L., Wender, D. B., Sloan, J. A., Dalton, R. J., Balcueva, E. P., Atherton, P. J., Bernath Jr, A.M., DeKrey, W.L., Larson, T., Bearden III, J.D., Carpenter, P.C., Loprinzi, C. L., 2007. Randomized controlled trial to evaluate transdermal testosterone in female cancer survivors with decreased libido; North Central Cancer Treatment Group protocol N02C3. J. Natl. Cancer Inst., 99(9), 672-679.  
  
Barton, M., 2012. Position paper: the membrane estrogen receptor GPER– Clues and questions. Steroids 77(10), 935-942.  
  
Basson, R., 2010. Review: testosterone therapy for reduced libido in women. Ther. Adv. Endocrinol. Metab. 1(4), 155-164.  
  
Beaton, E.A., Khan, A., deCatanzaro, D., 2006. Urinary sex steroids during sexual development in female mice and in proximate novel males. Horm. Metab. Res. 38, 501–506.  
  
Bellringer, J.F., Pratt, H.P.M., Keverne, E.B., 1980. Involvement of the vomeronasal organ and prolactin in pheromonal induction of delayed implantation in mice. J. Reprod. Fertil. 59, 223–228.  
  
Bensafi, M., Brown, W. M., Khan, R., Levenson, B., Sobel, N., 2004. Sniffing human sex-steroid derived compounds modulates mood, memory and autonomic nervous system function in specific behavioral contexts. Behav. Brain Res., 152(1), 11-22.  
  
Bensafi, M., Brown, W.M., Tsutsui, T., Mainland, J.D., Johnson, B.N., Bremner, E.A., Young, N., Mauss, I., Ray, B., Gross, J., Richards, J., Stappen, I., Levenson, R.W., Sobel, N., 2003. Sex-steroid derived compounds induce sex-specific effects on autonomic nervous system function in humans. Behav. Neurosci. 117(6), 1125-1134.  
  
Bliss, E. L., Frischat, A., Samuels, L., 1972. Brain and testicular function. Life Sci. 11(5), 231-238.  
  
Bogaert, A.F., 2005. Age at puberty and father absence in a national probability sample. J. Adolescence 28(4), 541-546.  
  
Booth, J. E., 1977. Sexual behaviour of neonatally castrated rats injected during infancy with oestrogen and dihydrotestosterone. J. Endocrinol., 72(2), 135-141.  
  
Bronson, F.H., 1975. Male-induced precocial puberty in female mice: confirmation of the role of estrogen. Endocrinology 96, 511‒514.   
  
Bronson, F.H., Desjardins, C., 1974. Circulating concentrations of FSH, LH, estradiol and progesterone associated with acute male-induced puberty in female mice. Endocrinology 94, 1658–1668.   
  
Bronson, F.H., Stetson, M.H., 1973. Gonadotrophin release in prepubertal female mice following male exposure: a comparison with an adult cycle. Biol. Reprod. 9, 449–459.   
  
Bronson, F.H., Whitten, W.K., 1968. Oestrus-accelerating pheromone of mice: assay, androgen-dependency and presence in bladder urine. J. Reprod. Fertil. 15, 131–134.  
  
Brooksbank, B.W., 1970. Labelling of steroids in axillary sweat after administration of 3H-delta5-pregnenolone and 14 C-progesterone to a healthy man. Experientia 26, 1012-1014.  
  
Bruce, H.M., 1960. A block to pregnancy in the mouse caused by proximity of strange males. J. Reprod. Fertil. 1, 96–103.  
  
Bruce, H.M., 1965. Effect of castration on the reproductive pheromones of male mice. J. Reprod. Fertil. 10, 141–143.  
  
Burnham, T.C., Chapman, J.F., Gray, P.B., McIntyre, M.H., Lipson, S.F., Ellision, P.T., 2003. Men in committed, romantic relationships have lower testosterone. Horm. Behav. 44, 119-122.  
  
Cheshenko, K., Pakdel, F., Segner, H., Kah, O., Eggen, R.I., 2008. Interference of endocrine disrupting chemicals with aromatase CYP19 expression or activity, and consequences for reproduction of teleost fish. Gen. Comp. Endocr. 155(1), 31-62.  
  
Cutler, W.B., Garcia, C.R., and Kreiger, A.M., 1979. Sexual behavior frequency and menstrual cycle length in mature premenopausal women. Psychoneuroendocrinology 4, 297-309.  
  
Cutler, W.B., Preti, G., Erickson, B., Huggins, G.R., and Garcia, C.R., 1985. Sexual behavior frequency and biphasic ovulatory type menstrual cycles. Physiol. Behav. 34(5), 805-810.  
  
Cutler W.B., Preti, G., Kriger, A., Huggins, G.R., Garcia, C.R., Lawley, H.J., 1986. Human axillary secretions influence women's menstrual cycles: the role of donor extract from men. Horm. Behav. 20, 463-473.  
  
Dabbs, J. M., 1990. Age and seasonal variation in serum testosterone concentration among men. Chronobiol. Int. 7(3), 245-249.  
  
Davis, S. R., & Braunstein, G. D., 2012. Efficacy and safety of testosterone in the management of hypoactive sexual desire disorder in postmenopausal women. J. Sex. Med. 9(4), 1134-1148.  
  
Davis, S. R., Goldstat, R., Papalia, M. A., Shah, S., Kulkarni, J., Donath, S., & Bell, R. J., 2006. Effects of aromatase inhibition on sexual function and well-being in postmenopausal women treated with testosterone: a randomized, placebo-controlled trial. Menopause 13(1), 37-45.  
  
deCatanzaro, D., 1987. Inhibition of estrogen-induced lordosis through central administration of corticosterone in adrenalectomized-ovariectomized rats. Neuroendocrinology 46, 468–474.  
  
deCatanzaro, D., 1988. Effect of predator exposure upon early pregnancy in mice. Physiol. Behav. 43, 691–696.  
  
deCatanzaro, D., 1999. Motivation and emotion: Evolutionary, physiological, developmental, and social perspectives. New Jersey: Prentice Hall.  
  
deCatanzaro, D., 2015. Sex steroids as pheromones in mammals: the exceptional role of estradiol. Horm. Behav. 68, 103-116.  
  
deCatanzaro, D., Baptista, M.A.S., Vella, E.S., 2001. Administration of minute quantities of 17β-estradiol on the nasal area terminates early pregnancy in inseminated female mice. Pharmac. Biochem. Behav. 69, 503–509.  
  
deCatanzaro, D., Beaton, E.A., Khan, A., Vella, E., 2006. Urinary oestradiol and testosterone levels from novel male mice approach values sufficient to disrupt early pregnancy in nearby inseminated females. Reproduction 132, 309–317.  
  
deCatanzaro, D., Graham, C., 1992. Influence of exogenous epinephrine on two reproductive parameters in female mice: disruption of receptivity but not implantation. Horm. Behav. 26, 330–338.

deCatanzaro, D., Khan, A., Berger, R.G., Lewis, E., 2009. Exposure to developing females induces polyuria, polydipsia, and altered urinary levels of creatinine, 17β-estradiol, and testosterone in adult male mice (*Mus musculus*). Horm. Behav. 55, 240–247  
  
deCatanzaro, D., MacNiven, E., Ricciuti, F., 1991. Comparison of the adverse effects of adrenal and ovarian steroids on early pregnancy in mice. Psychoneuroendocrinology 16, 525–536.  
  
deCatanzaro, D., Muir, C., O'Brien, J., Williams, S., 1995b. Strange-male-induced pregnancy disruption in mice: reduction of vulnerability by 17β-estradiol antibodies. Physiol. Behav. 58, 401–404.  
  
deCatanzaro D, Pollock T, Greville LJ, Faure PA., 2014. Estradiol transfer from male big brown bats (*Eptesicus fuscus*) to the reproductive and brain tissues of cohabiting females, and its action as a pheromone. Gen. Comp. Endocr. 208, 126-133.  
  
deCatanzaro, D., Storey, A., 1989. Partial mediation of strange-male-induced pregnancy blocks by sexual activity in mice (*Mus musculus*). J. Comp. Psychol. 13, 381–388.  
  
deCatanzaro, D., Smith, M., Muir, C., 1995a. Strange-male-induced pregnancy disruption in mice: potentiation by administration of 17β-estradiol to castrated males. Physiol. Behav. 58, 411–414.  
  
Delville, Y., Mansour, K.M., Ferris, C.F., 1996. Testosterone facilitates aggression by modulating vasopressin receptors in the hypothalamus. Physiol. Behav. 60, 25-29.  
  
Dulac, C., Torello, A.T., 2003. Molecular detection of pheromone signals in mammals: from genes to behavior. Nat. Rev. Neurosci. 4, 551–562.  
  
Ellis, B.J., Garber, J., 2000. Psychosocial antecedents of variation in girls' pubertal timing: maternal depression, stepfather presence, and marital and family stress. Child Dev. 71(2), 485-501.  
  
Feder, H. H., Whalen, R. E., 1965. Feminine behavior in neonatally castrated and estrogen-treated male rats. Science, 147(3655), 306-307.  
  
Ferrini, R.L., Barrett-Connor, E., 1998. Sex hormones and age: a cross-sectional study of testosterone and estradiol and their bioavailable fractions in community-dwelling men. Am. J. Epidemiol. 147(8), 750-754.  
  
Fritsch M., Orfanos C.E., Zouboulis C.C., 2001. Sebocytes are the key regulators of androgen homeostasis in human skin. J Invest. Dermatol., 116, 793-800.  
  
Gorski, R.A., 1993. Editorial: estradiol acts via the estrogen receptor in the sexual differentiation of the rat brain, but what does this complex do? Endocrinology 133, 431-432.  
  
Gower, D.B., Holland, K.T., Mallet, A.I., Rennie, P.J., Watkins, W.J., 1994. Comparison of 16-androstene steroid concentrations in sterile apocrine sweat and axillary secretions: interconversions of 16-androstenes by the axillary microflora—a mechanism for axillary odour production in man? J. Steroid Biochem. Mol. Biol. 48(4), 409-418.  
  
Green, R., Luttge, W.G., Whalen, R.E., 1967. Induction of receptivity in ovariectomized female rats by a single intravenous injection of estradiol-17β. Physiol. Behav. 5(2), 137-141.  
  
Guay, A. T., Jacobson, J., 2002. Decreased free testosterone and dehydroepiandrosterone-sulfate (DHEA-S) levels in women with decreased libido. J. Sex Marital Ther. 28(S1), 129-142.  
  
Guzzo, A.C., Berger, R.G., deCatanzaro, D., 2010. Excretion and binding of tritium-labelled oestradiol in mice (*Mus musculus*): implications for the Bruce effect. Reproduction 139, 255-263.  
  
Guzzo, A.C., Jheon, J., Imtiaz, F., deCatanzaro, D., 2012. Oestradiol transmission from males to females in the context of the Bruce and Vandenbergh effects in mice (*Mus musculus*). Reproduction 143, 539–548.  
  
Guzzo, A.C., Pollock, T., deCatanzaro, D., 2013. Transfer of [3H]estradiol-17β and [3H]progesterone from conspecifics to cohabiting female mice. J. Endocrinol. 217, 1–10*.*  
  
Harder, J.D., Jackson, L.M., 2003. Male pheromone stimulates ovarian follicular development and body growth in juvenile female opossums (*Monodelphis domestica*). Reprod. Biol. Endocrinol. 1, 21.  
  
Hasler, M.J., Nalbandov, A.V., 1974. The effect of weanling and adult males on sexual maturation in female voles (*Microtus ochrogaster*). Gen. Comp. Endocr. 23(2), 237-238.  
  
Hays, W.S.T., 2003. Human pheromones: have they been demonstrated? Behav. Ecol. Sociobiol. 54, 89-97.  
  
Hunt, J.S., Petroff, M.G., Burnett, T.G., 2000. Uterine leukocytes: key players in pregnancy. Semin. Cell Dev. Biol. 11, 127–137.  
  
Izard, M.K., Vandenbergh, J.G., 1982. The effects of bull urine on puberty and calving date in crossbred beef heifers. J. Anim. Sci. 55, 1160–1168.  
  
Jackman, P.J.H., Noble, W.C., 1983. Normal axillary skin microflora in various populations. Clin. Exp. Dermatol. 8(3), 259-268.  
  
Jacob, S., Hayreh, D.J., McClintock, M.K., 2001. Context-dependent effects of steroid chemosignals on human physiology and mood. Physiol. Behav. 74(1), 15-27.  
  
Kimura, N., Mizokami, A., Oonuma, T., Sasano, H., Nagura, H., 1993. Immunocytochemical localization of androgen receptor with polyclonal antibody in paraffin-embedded human tissues. J. Histochem. Cytochem. 41(5), 671-678.  
  
Kohl, J. V., Atzmueller, M., Fink, B., Grammer, K., 2001. Human pheromones: integrating neuroendocrinology and ethology. Neuroendocrinol. Lett. 22(5), 309-321.  
  
Kraemer, H. C., Becker, H. B., Brodie, H. K. H., Doering, C. H., Moos, R. H., Hamburg, D. A., 1976. Orgasmic frequency and plasma testosterone levels in normal human males. Arch. Sex. Behav. 5(2), 125-132.  
  
Kuiper, G.G., Carlsson, B., Grandien, K., Enmark, E., Häggblad, J., Nilsson, S., Gustafsson, J.-Å., 1997. Comparison of the ligand binding specificity and transcript tissue distribution of estrogen receptors α and β. Endocrinology 138, 863–870.  
  
Lazaros, L., Xita, N., Kaponis, A., Hatzi, E., Plachouras, N., Sofikitis, N., Zikopoulos, K.,Georgiou, I., 2011. The association of aromatase (CYP19) gene variants with sperm concentration and motility. Asian J. Androl. 13(2).  
  
Leung, K.C., Johannsson, G., Leong, G.M., Ho, K.K.Y., 2004. Estrogen regulation of growth hormone action. Endocrinology 25, 693–721.

Lu, A.P., Beehner, J.C., 2012. A Vandenbergh effect in wild geladas? The 81st meeting of the American Association of physical anthropologists. Retrieved from <http://meeting.physanth.org/program/2012/session15/lu-2012-a-vandenbergh-effect-in-wild-geladas.html>.   
  
Ma, W., Song, H., Das, S.K., Paria, B.C., Dey, S.K., 2003. Estrogen is a critical determinant that specifies the duration of the window of uterine receptivity for implantation. Proc. Nat. Acad. Sci. U.S.A. 100, 2963–2968.  
  
Macrides, F., Bartke, A., Dalterio, S., 1975. Strange females increase plasma testosterone levels in male mice. Science, 189(4208), 1104-1106.  
  
Marsden, H.M., Bronson, F.H., 1964. Estrous synchrony in mice: alteration by exposure to male urine. Science 144, 3625.   
  
Mazor, M., Hershkovitz, R., Chaim, W., Levy, J., Sharony, Y., Leiberman, J.R., Glezerman, M., 1994. Human preterm birth is associated with systemic and local changes in progesterone/17β-estradiol ratios. Am. J. Obstet. Gynecol. 171(1), 231-236.  
  
McClintock, M.K., 1971. Menstrual synchrony and suppression. Nature 229, 244-245.  
  
McEwan, I.J., 2004. Molecular mechanisms of androgen receptor-mediated gene regulation: structure-function analysis of the AF-1 domain. Endocr.-Relat. Cancer 11(2), 281-293.  
  
McGrady, A.V., 1984. Effects of psychological stress on male reproduction: a review. Syst. Biol. Reprod. Med. 13(1), 1-7.  
  
McGrath, K.G., 2009. Apocrine sweat gland obstruction by antiperspirants allowing transdermal absorption of cutaneous generated hormones and pheromones as a link to the observed incidence rates of breast and prostate cancer in the 20th century. Med. Hypotheses 72(6), 665-674.  
  
Monti‐Bloch, L., Jennings‐White, C., Berliner, D.L., 1998. The human vomeronasal system: a review. Ann. N. Y. Acad. Sci., 855(1), 373-389.  
  
Muir, C.C., Treasurywala, K., McAllister, S., Sutherland, J., Dukas, L., Berger, R.G., Khan, A., deCatanzaro, D., 2008. Enzyme immunoassay of testosterone, 17β-estradiol, and progesterone in perspiration and urine of preadolescents and young adults: exceptional levels in men’s axillary perspiration. Horm. Metab. Res., 40, 819-826.  
  
Nilsson, S., Mäkelä, S., Treuter, E., Tujague, M., Thomsen, J., Andersson, G., Enmark, E., Pettersson, K., Warner, M., Gustafsson, J.Å., 2001. Mechanisms of estrogen action. Physiol. Rev. 81(4), 1535-1565.  
  
Ogasawara, Y., Okamoto, S., Kitamura, Y., Matsumoto, K., 1983. Proliferative pattern of uterine cells from birth to adulthood in intact, neonatally castrated, and/or adrenalectomized mice, assayed by incorporation of [125I] iododeoxyuridine. Endocrinology 113, 582–587.  
  
Ortiz, M.E., Villalon, M., Croxatto, H.B., 1979. Ovum transport and fertility following postovulatory treatment with estradiol in rats. Biol. Reprod. 21, 1163–1167.  
  
Parkes, A.S., Bruce H.M., 1962. Pregnancy block in female mice placed in boxes soiled by males. J. Reprod. Fertil. 4, 303–308.  
  
Pfaff, D.W., 1980. Estrogens and brain function: Neural analysis of a hormone-controlled mammalian reproductive behavior. Springer-Verlag: New York.

Pielecka-Fortuna, J., Chu, Z., Moenter, S.M., 2008. Kisspeptin acts directly and indirectly to increase gonadotropin-releasing hormone neuron activity and its effects are modulated by estradiol. Endocrinology 149(4), 1979-1986.  
  
Potter, S.W., Gaza, G., Morris, J.E., 1996. Estradiol induces E-cadherin degradation in mouse uterine epithelium during the estrous cycle and early pregnancy. J. Cell. Physiol. 169(1), 1-14.  
  
Preti, G., Wysocki, C.J., Barnhart, K.T., Sondeimer, S.J., Leyden, J.J., 2003. Male axillary extracts contain pheromones that affect pulsatile secretion of luteinizing hormone and mood in women recipients. Biol. Reprod., 68, 2107-2113.  
  
Rajabi, N., Thorpe, J.B., Foster, W.G., deCatanzaro, D., 2014. Novel male exposure reduces uterine e-cadherin, increases uterine luminal area, and diminishes progesterone levels while disrupting blastocyst implantation in inseminated mice. J. Steroid Biochem. Mol. Biol. 139, 107–113.  
  
Rantala, M. J., Eriksson, C. J., Vainikka, A., Kortet, R., 2006. Male steroid hormones and female preference for male body odor. Evol. Hum. Behav., 27(4), 259-269.  
  
Reasner, D.S., Johnston, R.E., 1988. Acceleration of reproductive development in female Djungarian hamsters by adult males. Physiol. Behav. 43, 57–64.  
  
Reddy, V.V.R., Naftolin, F., Ryan, K.J., 1974. Conversion of androstenedione to estrone by neural tissues from fetal and neonatal rat. Endocrinology 94, 117-121.  
  
Reinberg, A., Lagouguey, M., Chauffournier, J.M., Cesselin, F., 1975. Rhythmes annuels et circadiends de la testosterone plasmatique chez cinq parisiens jeunes, adultes et sains. Ann. Endocrinol. - Paris 36, 44-45.  
  
Roberts, E.K., Lu, A., Bergman, T.J., Beehner, J.C., 2012. A Bruce effect in wild geladas. Science 335, 1222–1225.  
  
Rothardt, G., Beier, K., 2001. Peroxisomes in the apocrine sweat glands of the human axilla and their putative role in pheromone production. Cell. Mol. Life Sci. 58, 1344–1349.

Rupp, H.A., Wallen, K., 2007. Relationship between testosterone and interest in sexual stimuli: the effect of experience. Horm Behav. 52(5), 581-589.  
  
Sato, K., Leidal, R., Sato, F., 1987. Morphology and development of an apoeccrine sweat gland in human axillae. Am. J. Physiol.- Reg. I. 252(1), R166-R180.  
  
Sato, T., Wang, G., Hardy, M., Kurita, T., Cunha, G.R., Cooke, P., 2002. Role of systemic and local IGF-1 in the effects of estrogen on growth and epithelial proliferation of mouse uterus. Endocrinology 143(7), 2673–2679.  
  
Savic, I., Berglund, H., Gulyas, B., Roland, P., 2001. Smelling of odorous sex hormone-like compounds causes sex-differentiated hypothalamic activations in humans. Neuron 31(4), 661-668.  
  
Sawaya, M.E., Price, V.H., 1997. Different levels of 5alpha-reductase type I and II, aromatase, and androgen receptor in hair follicles of women and men with androgenetic alopecia. J. Invest. Dermatol. 109(2), 96-300.  
  
Shelley, W.B., Hurley, H.J., 1953. The physiology of the human axillary apocrine sweat gland. J. Invest. Dermatol. 20(4), 285-297.  
  
Siegelmann-Danieli, N., Buetow, K.H., 1999. Constitutional genetic variation at the human aromatase gene (Cyp19) and breast cancer risk. Br. J. Cancer 79(3-4), 456.  
  
Simerly, R.B., Chang, C., Muramatsu, M., Swanson, L.W., 1990. Distribution of androgen and estrogen receptor mRNA-containing cells in the rat brain: an in situ hybridization study. J. Comp. Neurol. 294, 76–95.  
  
Simpson, E.R., Davis, S.R., 2001. Minireview: aromatase and the regulation of estrogen biosynthesis—some new perspectives. Endocrinology 142(11), 4589-4594.  
  
Stern, K., McClintock, M.K., 1998. Regulation of ovulation by human pheromones. Nature 392(6672), 177-179.  
  
Stewart, M.E., Downing, D.T., Cook, J.S., Hansen, J.R., Strauss, J.S., 1992. Sebaceous gland activity and serum dehydroepiandrosterone sulfate levels in boys and girls. Arch. Dermatol. 128(10), 1345.  
  
Takayasu, S., Wakimoto, H., Itami, S., Sano, S., 1980. Activity of testosterone 5

alpha-reductase in various tissues of human skin . J. Invest. Dermatol. 74, 187-191.  
  
Thornton, M.J., Nelson, L.D., Taylor, A.H., Birch, M.P., Laing, I., Messenger, A.G., 2006. The modulation of aromatase and estrogen receptor alpha in cultured human dermal papilla cells by dexamethasone: a novel mechanism for selective action of estrogen via estrogen receptor beta? J. Invest. Dermatol. 126, 2010-2018.  
  
Thorpe, J.B., Burgess, P.S., Sadkowski, M., deCatanzaro, D., 2013. Estrogen-progesterone balance in the context of blastocyst implantation failure induced by predator stress. Psychoneuroendocrinology 38, 3048–3056.  
  
Thorpe, J.B., deCatanzaro, D., 2012. Oestradiol treatment restores the capacity of castrated males to induce both the Vandenbergh and the Bruce effects in mice (*Mus musculus*). Reproduction 143, 123-132.  
  
Thorpe, J.B., Gould, K.E., Borman, E.D., deCatanzaro, D., 2014. Circulating and urinary adrenal corticosterone, progesterone, and estradiol in response to acute stress in female mice (*Mus musculus*). Horm. Metab. Res. 46, 211–218.  
  
Tibbetts, T.A., Conneely, O.M., O'Malley, B.W., 1999. Progesterone via its receptor antagonizes the pro-inflammatory activity of estrogen in the mouse uterus. Biol. Reprod. 60, 1158–1165.  
  
Trotier, D., Eloit, C., Wassef, M., Talmain, G., Bensimon, J. L., Døving, K. B., Ferrand, J., 2000. The vomeronasal cavity in adult humans. Chem. Senses 25(4), 369-380.  
  
Türker, S., Onur, E., Ózer, Y., 2004. Nasal route and drug delivery systems. Pharm. World Sci. 26, 137‒142.  
  
Van Pottelbergh, I., Goemaere, S., Kaufman, J.M., 2003. Bioavailable estradiol and an aromatase gene polymorphism are determinants of bone mineral density changes in men over 70 years of age. J. Clin. Endocrinol. Metab. 88(7), 3075-3081.  
  
Vandenbergh, J.G., 1967. Effect of the presence of a male on the sexual maturation of female mice. Endocrinology 81(2), 354–349.  
  
Veith, J.L., Buck, M., Getzlaf, S., Van Dalfsen, P., Slade, S., 1983. Exposure to men influences the occurrence of ovulation in women. Physiol. Behav. 31(3), 313-315.  
  
Waxenberg, S.E., Drellich, M.G., Sutherland, A.M., 1959. The role of hormones in human behavior. I. Changes in female sexuality after adrenalectomy. J. Clin. Endocrinol. Metab. 19(2), 193-202.  
  
Whitten, W.K., 1956. Modification of the oestrous cycle of the mouse by external stimuli associated with the male. J. Endocrinol. 13, 399‒404.   
  
Whitten, W.K., 1958. Modification of the oestrous cycle of the mouse by external stimuli associated with the male. Changes in the oestrous cycle determined by vaginal smears. J. Endocrinol. 17, 307–313.   
  
Wilke, K., Martin, A., Terstegen, L., Biel, S.S., 2007. A short history of sweat gland biology. Int. J. Cosmetic Sci. 29(3), 169-179.  
  
Wilson, H.C., 1992. A critical review of menstrual synchrony research. Psychoneuroendocrinology 17(6), 565-591.  
  
Wingfield, J.C., Hegner, R.E., Dufty, A.M., Ball, G.F., 1990. The “challenge hypothesis”: theoretical implications for patterns of testosterone secretion, mating systems, and breeding strategies. Amer. Nat. 136(6), 829-846.  
  
Wysocki, C.J., Preti, G., 2004. Facts, fallacies, fears, and frustrations with human pheromones. Anat. Rec. A Discov. Mol. Cell. Evol. Biol. 281(1), 1201-1211.

Zouboulis, C.C., Chen, W.C., Thornton, M.J., Qin, K., Rosenfield, R., 2007. Sexual hormones in human skin. Horm. Metab. Res. 39, 85-95.

6.0. Appendices  
 **6.1. Appendix A: Recruitment Poster**

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**6.2. Appendix B: Consent Form**

DATE: \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_  
PARTICIPANT ID: \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_  
RESEARCHER: \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

17β-Estradiol levels in axillary perspiration of adolescent males:   
Environmental and genetic factors   
  
LETTER OF INFORMATION / CONSENT

Investigators:

Principal Investigator/ Faculty Supervisor:

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Research Sponsor: Natural Sciences and Engineering Research Council of Canada (NSERC)

Purpose of the Study: This study is being conducted for a Master's thesis. The aims are 1) to develop a new, inexpensive method of perspiration collection that can be used to measure levels of naturally occurring steroid hormones in human perspiration, 2) to assess intra-individual variation in such steroid levels (especially estradiol) in axillary (underarm) perspiration, and 3) to assess familial concordance of such steroid levels (i.e. to what extent estradiol levels correlate between family members).

You are invited to take part in this study on steroid hormone levels in human perspiration. We are hoping to find out the relative contribution of environmental and genetic factors to levels of estradiol in human axillary perspiration. This study aims to specifically investigate estradiol levels in men, because we believe that it may act as a chemical messenger (i.e. that it may enable males' perspiration to cause physiological effects in females' bodies).

Procedures involved in the Research: If you choose to volunteer to participate in the study, you will be asked to answer a paper questionnaire. You will also be asked to provide a perspiration sample. To promote perspiration, you may be asked to perform simple exercises after you fill out a short questionnaire ensuring your health and ability to exercise. Your time commitment for today will be approximately 1 hour.  
  
You may be invited to return to our lab to do the same testing at a different date. You may also be asked to provide contact information for your father and/or brother(s) (if applicable) so that we may contact them to ask whether they would be willing to participate in this study. If you do provide us with contact information for your father and/or brother(s), they will know that you have participated in this study, but the data we collect from you will not be disclosed to them in any way.   
  
Your visit today will take approximately 1 hour if you decide to fill out the paper questionnaire and provide a perspiration sample.

Potential Harms, Risks or Discomforts: If you choose to volunteer to participate in this study, the questionnaire which you will be asked to fill out will ask some personal questions about sexual relationships and lifestyle habits. For example, it will ask whether you prefer to date men, women, or both. Disclosing such information may provide a social risk for you, but we assure you that we will not share your questionnaire answers with anyone.

If you feel uncomfortable performing any part of the study, you can terminate your participation completely. Also, while we encourage you to complete all aspects of the study, you can simply leave questions blank or choose not to give a perspiration sample. Privacy will be provided while collecting perspiration samples, and while exercising if this is required to get these samples. You will be permitted to change out of your street clothes before you exercise if you wish. Perspiration samples will be discarded using standard protocol once they are analyzed in our lab.

Potential Benefits: We hope to achieve a greater understanding of the ways in which genetic and environmental factors affect steroids levels, and ultimately about how such steroids could potentially affect other humans via pheromonal action. Pheromones are chemical messengers that can create physiological affects between members of the same species. If you have any questions or would like to learn more about this line of research, you can feel free to ask the researcher.

Reimbursement: You will receive $12 for filling out the paper questionnaire and providing a perspiration sample. If you are asked to return to the lab to donate another perspiration sample, you will receive $5 for subsequent visits, as the time commitment will be much shorter.

Confidentiality: You are participating in this study confidentially. Your contact information will only be kept for the purposes of reaching you to schedule visits to the lab. The only link between your participant ID number and your identity is a database that is kept on a password-protected computer in a locked office. Your questionnaires will also be kept in a locked office. Only the student investigator(s) will ever know you by name, and we will share your contact information with no one else. Dr. deCatanzaro, or anyone who may analyze the data, will never know your identity. Furthermore, we will not know that the answers you provide on the questionnaire are your answers because we will only see your participant ID number when we read your questionnaire. Lastly, we will never disclose any individual data gathered though this study; we will report only group means.Once the study is complete, an archive of the data, without identifying information, will be maintained indefinitely.

Participation and Withdrawal: Your participation in this study is voluntary. If you decide to be part of the study, you can withdraw from the study for whatever reason, even after signing the consent form or after leaving the lab today (until approximately June 2014). If you decide to withdraw, there will be no consequences to you. In cases of withdrawal, any data you have provided will be destroyed unless you indicate otherwise. If you do not want to answer some of the questions you do not have to, but you can still be in the study. You will still be compensated if you choose to withdraw.

Information about the Study Results: We expect to have this study completed by approximately June 2014*.* If you would like a brief summary of the results, please let us know how you would like it sent to you.

Questions about the Study: If you have questions or would like more information about the study itself, please contact us at:

steroidstudymac@outlook.com

This study has been reviewed by the McMaster University Research Ethics Board and received ethics clearance. If you have concerns or questions about your rights as a participant or about the way the study is conducted, please contact:

McMaster Research Ethics Secretariat

Telephone: (905) 525-9140 ext. 23142

c/o Research Office for Administrative Development and Support

E-mail: ethicsoffice@mcmaster.ca

CONSENT

I have read the information presented in the information letter about a study being conducted by Brittney Elliott and Dr. Denys deCatanzaro of McMaster University.

I have had the opportunity to ask questions about my involvement in this study and to receive additional details I requested.

I understand that if I agree to participate in this study, I may withdraw from the study at any time or up until approximately June 2014.

I have been given a copy of this form.

I agree to participate in the study.

Signature: \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

Name of Participant (Printed) \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

1. Check either:  
  
[ ] I would like to receive a summary of the study’s results.

Please send them to this email address \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

Or to this mailing address: \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

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OR

[ ] No, I do not want to receive a summary of the study’s results.

2. I agree to be contacted about a follow-up visit, and understand that I can always decline the request.

[ ] Yes. Please contact me at: \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

[ ] No.

# 6.3. Appendix C: Questionnaire Participant ID: \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_ Date: \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

# Questionnaire 17β-Estradiol levels in axillary perspiration of adolescent males: environmental and genetic factors

*Please answer the following questions honestly, to the best of your ability. If you do not know the answer to a particular question, it's ok to write 'I don't know.'*

*1. How old are you?* \_\_\_\_\_\_\_\_\_\_ *2. Have you taken, in the past month,* ***any*** *medications, including prescription or non-prescription drugs?* YES NO  
 If so, what medication(s) have you taken? \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_  
 If you can't remember the name of the medication, what did you take it for? \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_  
  
*3. How many standard drinks (1 beer or 1 ounce of spirits or 5 ounces of wine) do you consume in the* ***average week****?* \_\_\_\_\_\_\_\_\_\_ *4. How many standard drinks (1 beer or 1 ounce of spirits or 5 ounces of wine) did you consume* ***last night****?* \_\_\_\_\_\_\_\_\_\_  
  
*5. In the last 24 hours:*  
 Have you eaten soy products, e.g. tofu/ soy milk/ soy beans/ edamame? YES NO  
 Have you consumed flax products, including flax seeds or bread with flax? YES NO  
  
*6. Do you eat, once a week or more:*  
 Soy milk? YES NO  
 Tofu? YES NO  
 Flax seeds? YES NO *7. How stressed have you been feeling over the past* ***month****, overall?*  
(circle one; 0 being not at all stressed and 5 being unbearably stressed)  
 0 1 2 3 4 5  
  
*8. How stressed have you been feeling over the past* ***week****?*  
(circle one; 0 being not at all stressed and 5 being unbearably stressed)  
 0 1 2 3 4 5  
  
*9. How many hours per week, on average, do you spend exercising (including exercises such as weightlifting, playing sports or jogging, but* ***not*** *including walking)?* (circle one)  
 0 1-3 4-6 7-9 10+ *10. Do you prefer to have romantic relationships with women or men?* (circle one)  
 1: exclusively men   
 2: mostly men   
 3: men or women   
 4: mostly women   
 5: exclusively women *11. Have you had sexual relations with a* ***female*** *in the last week?* YES NO  
 If so, was your partner taking hormonal birth control pills? YES NO  
  
*12. Have you had sexual relations with a* ***male*** *in the last week?* YES NO *13. Are you in a romantic relationship with a* ***female****?* YES NO If so:  
 For how long have you been dating your partner? \_\_\_\_\_\_\_\_\_\_  
 When is the last time your partner menstruated? \_\_\_\_\_\_\_\_\_\_   
 Is your partner taking hormonal birth control pills? YES NO  
 How frequently are you sexually active with your partner? \_\_\_\_\_\_\_\_\_\_  
  
*14. Are you in a romantic relationship with a* ***male****?* YES NO If so:  
 For how long have you been dating your partner? \_\_\_\_\_\_\_\_\_\_  
 How frequently are you sexually active with your partner? \_\_\_\_\_\_\_\_\_\_  
  
*15. Do you live with a romantic partner?* YES NO If so:  
 Is this person female or male? \_\_\_\_\_\_\_\_\_\_  
 If this person is female, is she taking hormonal birth control pills? YES NO  
 *16. Do you live with* ***any*** *other females, including familial relatives and non-relatives such as roommates?*

If so, please provide information for each one:

**Relationship Approximate Age How long have you lived together?**

\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

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**6.5. Appendix E: Debriefing Form**

17β-Estradiol levels in axillary perspiration of adolescent males:   
Environmental and genetic factors

DEBRIEFING FORM

Investigators:

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Research Sponsor: Natural Sciences and Engineering Research Council of Canada (NSERC)

Thank you for your participation in this study. As mentioned in the consent form, you have helped us in our investigation of the potential correlation between steroid content in human males' perspiration, genetic factors, and environmental variables such as stress levels, relationship status, and diet. On a grander scale, we suspect that estrogens in males' axillary perspiration could have pheromonal properties, and you have helped us get closer to understanding this issue.

What happens after you give us your sample(s)?

Each sample will be analyzed using an ELISA biochemical technique, which allows us to quantify the samples' steroid levels. Your samples will be measured for estrogens (specifically estradiol), and possibly other steroid hormones such as testosterone, progesterone. Statistical analyses will be conducted on the data, and trends in hormone levels will be compared with trends from the questionnaire. Perspiration samples will be disposed of using standard protocols at the end of the study.  
  
Where can you go if you were upset by any aspect of your participation in this study?If you feel upset about any aspect of your experience with us today (for example, the process of giving the perspiration sample or the questions on the questionnaire), McMaster University offers free counseling services through the Student Wellness Centre. You can find more information at: **http://wellness.mcmaster.ca/counselling.html**. Alternatively, you may phone to book an appointment at 905 525 9140, ext. 27700.

Confidentiality: You are participating in this study confidentially. Your contact information will only be kept for the purposes of reaching you to invite you to schedule visits to the lab. The only link between your participant ID number and your identity is a database that is kept on a password-protected computer in a locked office. Only the student investigator(s) will ever know you by name, and we will share your contact information with no one else. Dr. deCatanzaro, or anyone who may analyze data, will never know your identity. Furthermore, we will not know that the answers you provide on the questionnaire are your answers because we will only see your participant ID number when we read your questionnaire. Lastly, we will never disclose any individual data gathered though this study; we will report only group means. Once the study is complete, an archive of the data, without identifying information, will be maintained.

Participation and Withdrawal: Your participation in this study is voluntary. If you decide to be part of the study, you can decide to withdraw from the study for whatever reason, even after signing the consent form or after leaving the lab today (until approximately June 2014). If you decide to withdraw, there will be no consequences to you. In cases of withdrawal, any data you have provided will be destroyed unless you indicate otherwise. If you do not want to answer some of the questions you do not have to, but you can still be in the study. You will still be compensated if you choose to withdraw.

Information about the Study Results: We expect to have this study completed by approximately June 2014*.* If you would like a brief summary of the results, please let us know how you would like it sent to you.

Questions about the Study: If you would like to know more about this line of research, please use the contact information at the top of this page in order to reach our lab. Please note that results from this study will not be released until they are published.

If you have concerns or questions about your rights as a participant or about the way the study is conducted, please contact:

McMaster Research Ethics Secretariat

Telephone: (905) 525-9140 ext. 23142

c/o Research Office for Administrative Development and Support

E-mail: ethicsoffice@mcmaster.ca

Again, thank you for participating in this research. Without volunteers like you, certain advancements in scientific understanding would be impossible.

**6.6. Appendix F: Email Inquiry About Antiperspirant Use**  
  
The email sent to each participant to inquire about antiperspirant use read:  
  
*"Hello,  
Thank you again for participating, this past February-March, in our research involving levels of natural steroid hormones in men's axillary perspiration. We are emailing you today to request that you consider answering one brief question which we would like to add to the questionnaire you previously filled out. There is absolutely no obligation to provide this information, but if you have a moment, it would be helpful for you to do so. As always, any information you provide will be kept strictly confidential.  
The additional question we are interested in is:  
On average, how many times per week do you use antiperspirant on your underarms?  
A) I do not use antiperspirant or deodorants of any kind.  
B) I use all natural deodorant only.  
C) I use antiperspirant 1-2 days per week.  
D) I use antiperspirant 3-4 days per week.  
E) I use antiperspirant 5-7 days per week.  
If you are willing to provide this information, you may do so by replying to this email with your answer.  
Thank you so much for your time."*  
  
Note: For data analysis, this question was scored from 0-4, with A) receiving a score of 0 and E) receiving a score of 4.

**6.7. Appendix G: Residue From Filter Paper Extractions**

**Figure A:** Residue remaining in centrifuge tubes after filter paper extractions. This residue remained after the ethanol was completely evaporated, and likely contains sex steroids, salts, and other non-volatile components of perspiration. Image taken with a Samsung Galaxy S4 Mini phone camera.   
