DISTRESSED ABOUT THE STRESS RESPONSE:

ASSOCIATIONS BETWEEN HORMONAL CONTRACEPTIVE USE, WOMEN'S STRESS RESPONSE, INFLAMMATION, AND MOOD

by

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Submitted to the Graduate Faculty of the
College of Science and Engineering
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in partial fulfillment of the requirements
for the degree of

Doctor of Philosophy

May 2022

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For The College of Science and Engineering

ACKNOWLEDGEMENTS

This research was made possible through the support of a team of scientists, and I am very grateful for their contributions to my success. I would like to first acknowledge the key role of my academic advisor and dissertation chair, Dr. Sarah E. Hill. Her guidance on this project has vastly improved the project's research design, my academic writing, and my statistical interpretations. Beyond that, her years of mentorship have shaped me into the scientist I am today, and I will be forever grateful for her investment in me. I would also like to acknowledge and thank the other members of my dissertation committee, Dr. Gary Boehm, Dr. Naomi Ekas, Dr. Charlie Lord, and Dr. Brent Cooper. Dr. Naomi Ekas was particularly helpful in providing advice that improved the clarity of my writing. Additionally, Dr. Gary Boehm helped to shape my thinking about the neurological and immunological mechanisms that underlie the current research. His mentorship has allowed me to take my career in a direction I had never dreamed possible, and I am grateful for his many contributions to my personal and career development.

I would also like to thank my research assistants, Sally Ho, Jennie Chuah, Peyton McDuffee, Catherine Wise, Alexia Fornah, Emma Hultquist, Faith Zacharias, Caroline Davidson, Kennedy Redmon, Lexie Gray, Alexandra Dunker, Margaret Martin, Lauren Gorrebeeck, and Madison Herzog; they each played an integral part in running this research, and I would have been lost without them. I would also like give a special mention to Sally Ho, who played an essential role in managing the recruitment for this project, as her work was truly outstanding, alongside McKenzie Hawker, who managed the arduous data input necessary for this project with amendable accuracy and diligence. Further, I would like to thank my past and current colleagues, Dr. Randi Proffitt, Dr. Hannah K. Bradshaw, Dr. Jeff Gassen, Katja Cunningham, and Matthew Espinosa for their invaluable advice and constant support. Science is a team effort, and I would like to extend my full gratitude to each member of the team of scientists that helped this research project to come to fruition.

Beyond those who supported me academically, I would be remiss if I did not thank the many others who provided me with their love and support throughout this process. This includes my family, especially my parents, Rick and Gail Mengelkoch, and my siblings, Amber and Joe Eisenschenk, Thor and Rin Mengelkoch, April Mengelkoch, and Eric Mengelkoch. Finally, a special thanks to my partner, Brocke Addison, who provided feedback and encouragement at each step of this journey, and to my dog, Scrunchie, who made sure I always took a few minutes a day to smile and play.

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Distressed About the Stress Response: Associations Between Hormonal Contraceptive Use, Women's Stress Response, Inflammation, and Mood

Hormonal contraceptives (HCs) were first approved as a method of pregnancy prevention nearly sixty years ago (Bullough, 2001). Since then, HC use has increased dramatically, with more than 400 million women around the world using HCs (United Nations, 2019). In the United States, more than 80% of women report having used HCs during at least some of their reproductive-aged years (Daniels & Jones, 2013), with their use being the most common in those between the ages of 15-29 (Daniels & Abma, 2018).

Despite their widespread use, little is known about the downstream physiological and psychological side effects associated with HC use. That is, while there has been extensive research concerning HC safety and efficacy, far less research has been devoted to understanding the non-life-threatening physiological and behavioral consequences of HC use (for discussion, see Montoya & Bos, 2017; Pletzer & Kerschbaum, 2014). Emerging research, however, is revealing that HC use is associated with both structural and functional changes in the brain, including changes in brain regions associated with memory and emotional processing (e.g., Gingnell et al., 2013; Miedl et al., 2018; Person & Oinonen 2020; Petersen & Cahill, 2015; Pletzer et al., 2010, 2019). For example, in one study, researchers compared the impact of cortisol administration on implicit fear learning between men, women using HCs, and naturally cycling (NC) women (i.e., women not using any form of HCs). The researchers found that while cortisol administration reduced hippocampal fear learning in men and NC women, cortisol administration enhanced hippocampal fear learning in women using HCs (Merz et al., 2012). These results, when considered alongside of related work investigating how HC use impacts emotional memory processing (for review, see Lewis et al., 2019), indicate that women using

HCs may be more likely to attend to and remember negative, emotionally charged information when under stress than are men or NC women.

Beyond changes to the impact that cortisol has on neural processes, some research suggests that HC use is also associated with changes to the hypothalamic-pituitary-adrenal (HPA) axis-mediated stress response. Typically, when healthy adults experience an acute stressor, their levels of cortisol rise before returning to baseline levels following the conclusion of the stressful event. However, a growing body of research finds that for women using HCs, this cortisol response to acute stress is blunted (Kirschbaum et al., 1999; Nielsen et al., 2013) or altogether absent (Lovallo et al., 2019; Roche et al., 2013) relative to what is observed in men and NC women. In one of the first studies to document this effect, researchers exposed participants to the Trier Social Stress Task (TSST), a stress manipulation involving public speaking and mental math (Kirschbaum et al., 1999). The researchers measured cortisol levels and subjective ratings of the participants' stress levels before and after the stress task, and found women using HCs exhibited a cortisol response to stress that was less than half of the magnitude of the response exhibited by NC women, and even smaller compared to men's responses, despite the groups having no differences in baseline cortisol levels[1] prior to the stress task or subjective stress ratings following the task.

Cortisol plays an important neuro- and immuno-modulatory role throughout the body; as such, the possibility that women using HCs exhibit cortisol dysregulation is one that has a number of potential downstream physiological and psychological consequences. For example, cortisol plays a role in regulating blood sugar, fat distribution, and inflammation, meaning that women using HC could be at a greater risk of metabolic disorders, heightened abdominal fat

[1] Although others find women using HCs to exhibit elevated levels of cortisol (see e.g., Hertel et al., 2017).

distribution, and chronic systemic inflammation (Anagnostis et al., 2009; Björntorp & Rosmond, 2000; Hänsel et al., 2010). Emerging research finds women using HCs experience an elevated and extended morning cortisol peak compared to men and NC women (Lovallo et al., 2019). Further, research utilizing a multi-omics approach finds that women using HCs exhibit heightened levels of circulating cortisol, along with elevated triglycerides and decreased hippocampal volumes, in comparison to NC women (Hertel et al., 2017) – a physiological pattern that is strikingly similar to what is observed in animals which exhibit chronically elevated cortisol levels as the result of chronic and unpredictable stress exposures (Faria et al., 2014; Oliveira et al., 2016). In general, chronically heightened cortisol levels are associated with increases in one's risks for anxiety, depression, digestive problems, headaches, cardiovascular disease, sleep problems, weight gain, memory impairment, and type 2 diabetes (Guilliams & Edwards, 2010), and, while less explored, research suggests that a blunted cortisol response to acute stress is also associated with its own health risks (e.g., Phillips et al., 2013).

Another system that is profoundly affected by the activities of the HPA axis is the immune system, as cortisol plays an important role regulating the body's inflammatory response. Generally, people experience a rise in proinflammatory cytokines – signaling proteins which coordinate the body's inflammatory response – alongside a rise in cortisol following acute stress (Steptoe et al., 2007). One of the many functions of this rise in cortisol is to downregulate or modulate inflammatory processes, helping the body to return to normal following stress. Typically, cortisol and inflammatory responses to acute stress are inversely related, indicating that those who display a blunted cortisol response to stress may also display an increased inflammatory response to stress (Kunz-Ebrecht et al., 2003). Accordingly, in addition to a dysregulated cortisol response, women using HCs could exhibit dysregulated inflammatory

activity in response to acute psychosocial stress. However, women's inflammatory response to acute stress has yet to be characterized. This is particularly important as women taking HCs are reliably found to have higher levels of C-reactive protein (CRP), a marker of systemic inflammation, compared to NC women (Divani et al., 2015). Heightened CRP is a risk factor for many diseases of aging, including coronary heart disease. For example, in one study investigating the impact of hormone replacement therapy in post-menopausal women, researchers found that women receiving hormone replacement therapy had increased levels of CRP, and that those with the highest levels of CRP, after controlling for other factors known to increase risk for heart disease, were more than twice as likely to experience a cardiac event compared to those with the lowest levels of CRP (Pradhan et al., 2002). Given the role of inflammation in the pathogenesis of disease (Chung et al., 2009) and mood disorders, such as depression (Miller & Raison, 2016), dysregulated inflammatory responses to stress could have important implications for the mental and physical health of women using HCs.

Both elevated cortisol levels and elevated CRP levels, on their own, are associated with increased health risks, however, together, they have also been found to be associated with impaired cognitive performance. In one study, researchers found women with heightened hair cortisol concentrations performed worse on verbal working memory tasks compared to those with lower levels of hair cortisol concentrations (van den Heuvel et al., 2022). However, they also found that levels of CRP moderated relationships between cortisol levels and cognitive task performance. Specifically, while CRP levels were positively related to cognitive task performance for those with low levels of hair cortisol concentrations, CRP levels were negatively related to cognitive task performance in those with high levels of hair cortisol concentrations in tasks assessing language, verbal intelligence, and executive functioning. Results such as these

highlight that associations between HC use and both elevated cortisol and elevated CRP may have far-reaching consequences for women's cognitive function, in addition to their mental and physical health.

In the following, I present the results of a study examining the relationship between HC use (vs non-use) and women's cortisol and inflammatory responses to acute psychosocial stress. Guided by insights from existing work in this area, I predict that women taking HCs will exhibit a blunted cortisol and exaggerated inflammatory response to acute psychosocial stress when compared to NC women. Additionally, I examine whether the predicted differences in women's cortisol and inflammatory responses to stress are a) associated with differences in women's subjective appraisals of stress, b) associated with differences in women's moods following stress, and c) differ based upon the generation of progestins contained in the HCs that women are using. These latter analyses are exploratory in nature and seek to provide initial insight into the links between HCs, inflammation, and mood, and whether these relationships vary depending on type of HCs that women are using. This research will yield important new insights into how HC use may impact women's cortisol and inflammatory stress responses, both of which, if dysregulated, have implications for women's mental and physical health.

Hormonal Contraceptives

HCs come in many forms – an oral pill, a patch, an insertable ring, an injection, a subdermal implant, and an intrauterine device (IUD) – but all HCs are primarily composed of progestins. Progestins are a synthetic form of progesterone, which prevent pregnancy by binding to progesterone receptors in the body and suppressing ovulation (Bullough, 2001)[2]. One common

[2] Many oral HCs, known as combination pills, also contain a synthetic estrogen component, ethinyl estradiol.

way to categorize the many different types of HCs that women use is to group them into progestin generations, based on when the progestin contained in that method of HC came onto the market (see Table 1 for information about the different generations of progestins). Most progestins, rather than being derived from progesterone, are derived from testosterone (Sitruk-Ware, 2008).

Table 1

Generations of HC Progestins

	First Generation	Second Generation	Third Generation	Fourth Generation
Progestin Names	• Norethindrone/ Norethisterone acetate • Ethynodiol acetate • Medroxy-progesterone acetate	• Levonorgestrel • Norgestrel	• Desogestrel / Etonogestrel • Gestodene • Norgestimate	• Drospirenone • Dienogest
HC Types	• Oral HC • Hormonal therapy • Depo-Provera (injectable)	• Oral HC • Hormonal IUD • Emergency contraception	• Oral HC • Nexplanon (implant) Nuva Ring (insertable) • Hormone therapy	• Oral HC • Hormonal therapy
Androgenic Effects	• Variable in androgenicity – (low to moderate/highly androgenic)	• Highly androgenic	• Low androgenicity	• Anti-androgenic
Progestational Effects	• Moderate/highly progestational	• Highly progestational	• Highly progestational	• Little progestational activity

Note: gen = generation; HC = hormonal contraceptive. (Dickerson & Bucci, 2002; Edwards, 2004)

While natural progesterone only binds with cells which contain progesterone receptors, progestins contained in HCs are more promiscuous. Beyond binding with cells that contain progesterone receptors, most progestins also bind to cells with androgen receptors, glucocorticoid receptors, and mineralocorticoid receptors (Sitruk-Ware, 2008), indicating that progestins contained in HCs impact a wide variety of biological processes, beyond their intended function of suppressing ovulation. The affinity of progestins for binding with these different types of receptors could explain why some women experience HC-related side effects, such as weight gain, headaches, mood changes, decreased sexual desire, and acne (Barr, 2010), along with dysregulation of the stress response.

HCs and their Downstream Effects on Women's Psychology and Behavior

Beyond their role in preventing ovulation and pregnancy, HCs are found to have range of effects on women's psychology and behavior. Researchers find that, compared to NC women, women using HCs report decreased libido (Boozalis et al., 2016; Casey et al., 2017; Smith et al., 2014) and exhibit less perseverance on cognitive tasks (Bradshaw et al., 2020), less neural reactivity to viewing erotic stimuli (Abler et al., 2013), more neural reactivity to monetary rewards (Bonenberger et al., 2013), and differences in emotional processing and memory (Person & Oinonenm, 2020; Peteresen & Cahill, 2015). For example, in one double-blind, placebo-controlled, randomized study, researchers recruited participants who had experienced previous mood-related side effects of HC use and assigned half of these women to take a HC while the other half were assigned to take a placebo (Gingnell et al., 2013). After three weeks of HC use, women in the HC group reported more depressed mood, mood swings, and fatigue than did those in the placebo group. Additionally, when engaged in an emotion processing task, the HC group exhibited decreased reactivity in the left insula, left middle frontal gyrus, and bilateral inferior

frontal gyri compared to those in the placebo group, and a decrease in right amygdala reactivity compared to their own pre-treatment reactivity. While well-controlled studies such as these are the exception rather than the rule in much research investigating the impact of HC use on women's psychologies and behaviors, they provide initial evidence that HC use plays a causal role in influencing women's mood and neural function.

HCs and the Stress Response

To investigate how HC use could impact the HPA axis-mediated stress response, it is useful to understand the cascade of biological processes initiated by experiencing a stressful event, which are designed to help an organism to survive potentially threatening situations. Typically, stressful events activate the sympathetic nervous system and the HPA axis, which, together, help an organism to manage stressful situations and return the body to normal following stress (Bear et al., 2020). Within seconds of encountering a stressor, the sympathetic nervous system, known as the "fight or flight" response system, becomes activated, which leads to the release of epinephrine and norepinephrine. The release of these neurotransmitters increases heart rate, respiratory rate, sweat gland secretion, and mental alertness, while downregulating other processes, such as digestion, to conserve energetic resources while managing a potentially dangerous situation. Additionally, when the HPA axis becomes activated by stress, the hypothalamus releases corticotrophin-releasing hormone (CRH), which signals the anterior pituitary gland to release adrenocorticotropic hormone (ACTH). ACTH, in turn, stimulates adrenal glands, which then produce corticosteroid hormones, or cortisol. Cortisol helps the body to cope with stress by gathering readily available glucose stored in the liver and making it available in the bloodstream for use. Heightened and steady blood glucose levels allow an organism to expend energy managing potentially threatening situations and help the body to

return to normal after a stressful event is resolved. Additionally, cortisol inhibits the release of additional CRH and ACTH, downregulating the HPA axis-mediated stress response following its release.

Although stress affects men and women alike, researchers have only recently begun to include women in their studies investigating the biological stress response (e.g., Kirschbaum et al., 1999). Once women were included in this research, it became apparent that women taking HCs exhibit a blunted cortisol response to acute stressors compared to men and NC women (Kirschbaum et al., 1999; Lovallo et al., 2019; Nielsen et al., 2013; Roche et al., 2013). For example, in one study, Roche and colleagues (2013) utilized secondary data analysis to investigate differences in women's cortisol responses to the TSST and found that women taking HCs exhibited a severely blunted cortisol response following the stress task compared to NC women. Blunted cortisol reactivity to stressors has been found to be associated with many health issues including, for example, chronically heightened inflammation (e.g., Miller et al., 2008) and autoimmune disorders (e.g., atopic dermatitis; Buske-Kirschbaum et al., 1997), and to characterize smokers (e.g., al'Absi, 2006), those with substance addictions (e.g., Lovallo et al., 2000), and those with depression (e.g., Salomon et al., 2009).

While the first studies investigating the impact of HC use on cortisol responses to acute stressors were somewhat underpowered, findings have now been replicated in larger samples of women (Lovallo et al., 2019), using a variety of stress-inducing paradigms. To date, researchers report women using HCs to exhibit a blunted cortisol response to acute stress compared to NC women after exposure to social stress tasks (Kirschbaum et al., 1999; Lovallo et al., 2019; Roche et al., 2013), the socially evaluated cold-pressor test (Merz, 2017), the cold-pressor stress task (Nielsen et al., 2013), and to administered naltrexone, a drug that increases cortisol levels (Roche

et al., 2013). Extending these lines of research, Herrera and colleagues (2019) conducted the first study to better understand the differences in the cortisol responses to acute social stress in women depending upon the type of oral HC that participants were taking. The researchers found that women taking HCs containing second generation progestins exhibited a larger cortisol response to stress compared to women taking HCs containing first or third generation progestins, who displayed more blunted cortisol responses to acute stress. These results highlight the possibility that some types of HCs could have a stronger influence on women's cortisol response to stress than do others. Overall, mounting evidence that women who use HCs exhibit a blunted cortisol response to acute stress indicates that HC use likely impacts women's stress response systems, although the extent to which and the mechanisms by which this occurs, along with the downstream consequences of this dysregulation, are still being studied and debated.

HCs and the Inflammatory Stress Response. One potential mechanism by which dysregulation of the HPA axis-mediated stress response in women using HCs could have far reaching implications, is women's inflammatory response to stress. This is because, in addition to stimulating sympathetic nervous system and HPA axis activities, stress also stimulates the activities of the immune system, promoting the release of proinflammatory cytokines, which play a role in activating the HPA axis and are downregulated by cortisol release. Proinflammatory cytokines are signaling proteins which communicate between the central nervous system and immune system to produce and coordinate the inflammatory response, which is the body's first line of defense against injury, infection, and disease. Locally, inflammation increases blood flow and recruits white blood cells and phagocytes to combat threats and repair damaged tissue. More generally, throughout the body, inflammation coordinates a host of responses known as "sickness behavior" (Dantzer, 2001; Dantzer & Kelley, 2007), which include fever, fatigue, lack of

appetite, and anhedonia, all of which are theorized to promote decreased energy expenditure on non-essential tasks while the body is expending increased energy combatting pathogenic threats and repairing damaged tissue. Among the events known to prompt inflammatory events in the body is stress (Steptoe et al., 2007). Because stressful events in our evolutionary past were typically associated with a heightened risk of physical injury or disease, psychosocial stressors are found to be powerful stimulators of the inflammatory response. For example, research finds that levels of proinflammatory cytokines interleukin 1 beta (IL-1β), IL-6, and tumor necrosis factor alpha (TNF-α) each rise following stress, in both men and women, and in response to various forms of stress (Marsland et al., 2017; Slavish et al., 2015).

Given the relationships between inflammation and cortisol, it is possible that the dysregulation of the cortisol response to stress found in women using HCs is accompanied by a dysregulated inflammatory response to stress as well. While no studies have directly investigated the inflammatory responses to acute stress in women taking HCs, Rohleder and colleagues (2003) have investigated the impact of acute stress on the glucocorticoid (GC) sensitivity of stimulated proinflammatory cytokine release in women using HCs after stress *ex vivo* (i.e., in whole blood). Replicating previous work, they found that women taking HCs exhibited a blunted cortisol response to acute stress compared to NC women. Further, the researchers found that lipopolysaccharide (LPS) stimulated IL-6 production, in the absence of pharmacological (i.e., dexamethasone) inhibition, increased slightly and remained elevated following stress in women taking HCs, while stimulated IL-6 levels of NC women decreased immediately following the stress task and returned to baseline 60 minutes after the stress task[3], with this latter pattern being

[3]Rohleder and colleagues (2003) also found that women using HCs displayed increased GC sensitivity of proinflammatory cytokine release in response to stress, while this increase in GC sensitivity in response to stress was not found in NC women. Specifically, following stimulation with LPS, a lower dose of dexamethasone was needed to suppress IL-6 production by 50% in the blood cells of women taking HCs after the stress task than was

more similar to what is more typically observed (see e.g., Marsland et al, 2017). Although the interaction between group (HC vs NC) and time (pre-stress, post-stress, and 60 minutes post-stress) on stimulated IL-6 release did not reach statistical significance and this study was underpowered, casting some uncertainty as to the validity of these relationships, these results provide preliminary evidence that the dysregulation of the HPA axis in response to acute stress found in women taking HCs does not end with the cortisol response and may have further downstream physiological outcomes, such as an exaggerated inflammatory response to acute stress.

Inflammation and its Behavioral Sequelae. Inflammation, beyond its primary role of coordinating the immune response, is found to influence a host of neurobiological, cognitive, and behavioral processes (e.g., Dantzer, 2001; Dantzer & Kelley, 2007; Eisenberger et al., 2010; Hennessy et al., 2014; Jewett & Krueger, 2012; Moieni & Eisenberger, 2018). For example, in recent years, researchers have discovered overwhelming support for an association between depression and increased levels of inflammation (Capuron & Miller, 2011; Dantzer et al., 2008; Dooley et al., 2018; Miller & Raison, 2016; Raison & Miller, 2011). Beyond depression, researchers have reported relationships between increased inflammation and social disconnection (Eisenberger, et al., 2010), loneliness (Jaremka et al., 2013a, b), and anxiety (Moon et al., 2015; Moons & Shields, 2015). Further, experimentally raising one's levels of inflammation has been found to increase depressed mood (Reichenberg et al., 2001), and feelings of social disconnection (Eisenberger, et al., 2010), with the latter team of researchers finding

needed to achieve the same effect prior to the stress task, while this relationship was not found in NC women. These results imply that women taking HCs may have increased GC sensitivity of stimulated proinflammatory cytokine release in the context of acute stress, which the researchers argue may compensate for the low cortisol levels observed in these women in response to acute stressors. While intriguing, these preliminary results should be interpreted with caution, as researchers did not control for levels of cortisol in participants' blood prior to stimulation, which may have influenced their findings.

inflammation-induced depressed mood to be mediated by increased feelings of social disconnection.

The association between inflammation and depression is of keen interest, as HC use is also associated with depression (Bengtsdotter et al., 2018; Skovlund et al., 2016), and many women report mood-related side effects when using HCs, including depressive symptoms (Barr, 2010). However, results on the relationships between HC use and depression are inconsistent, as some researchers find women using HCs report fewer depressive symptoms and report more positive moods compared to NC women (Toffol et al., 2011; 2012). While it is likely true that HC treatment improves depressive symptoms for some women (e.g., Cheslack-Postava et al., 2015; Rasgon et al., 2003), for some periods of HC treatment (e.g., Lundin et al., 2017), it is clear that HC treatment is also associated with more depressive symptoms and more unpleasant moods in some women as well. The best evidence of this comes from a large, population-based study which investigated relationships between HC use and depression diagnoses and anti-depression treatment using the health records of over one million Danish women (Skovlund et al., 2016). These researchers found that previous HC use significantly increased women's risk of being both diagnosed with depression and being treated with anti-depressants, with younger women and women using non-oral HCs being at the highest risk for depression diagnoses. Given that results of research investigating associations between HC use and women's mood-related outcomes are mixed, it could be the case that researchers find these disparate results because the impact of HC use on depressive symptoms is mediated through one's inflammatory response to acute stress over time. That is, an exaggerated inflammatory response to stress due to HC-related HPA axis dysregulation could help to explain the observed links between HC use and depression, along with helping to explain the elevated levels of CRP found in women using HCs.

The Current Research

Researchers find that women taking HCs exhibit a blunted cortisol response to acute stress compared to NC women (Kirschbaum et al., 1999; Lovallo et al., 2019; Nielsen et al., 2013; Roche et al., 2013). While the results of these studies suggest that the stress response may become dysregulated in women taking HCs, much less work has examined the downstream consequences of this dysregulation, nor whether these are moderated by the types of progestins in the HCs women are using. The current research is aimed at redressing these gaps, with the goal of achieving the following three aims.

Aim 1

The first aim of the current project was to examine the impact of HC use on the cortisol and inflammatory response to psychosocial stress, which is a powerful immunological stimulant. This research is necessary because – despite the growing body of work demonstrating associations between HC use and the functioning of the HPA axis (see e.g., Kirschbaum et al., 1999; Lovallo et al., 2019; Nielsen et al., 2013; Roche et al., 2013) – very little is known about associations between HC use and the body's inflammatory response (for exception, see Rohleder et al., 2003). To achieve this aim, I exposed a large sample of women to psychosocial stress and examined their cortisol, inflammatory, and subjective responses to acute social stress. I predicted that, compared to NC women, women taking first through third generation oral HCs would exhibit a blunted cortisol and an exaggerated proinflammatory cytokine response to stress.

Aim 2

The second aim of the current research was to examine the links between HC use and a) women's subjective response to stress and b) its biological antecedents. Specifically, I investigated whether HC use moderated women's subjective responses to acute psychosocial

stressors. I predicted that, compared to NC women, women using HCs would report a more negative mood following stress. Further, I predicted that women's changes in mood following stress would be mediated through changes in levels of cortisol and/or proinflammatory cytokines resulting from exposure to the stressor. Specifically, I predicted that HC use (compared to non-use) would predict less dynamic changes in cortisol in response to the stressor and exaggerated inflammatory responses, both of which would predict a more negative mood following stress.

Aim 3

The final aim of the current project was to examine whether biological and subjective responses to stress in women using HCs vary as a function of the generation of progestin in the HC product they are using. Historically, researchers have treated all women taking HCs as one homogenous group, with little attention paid to the type of HC that women are using (for an exception, see Herrera et al., 2019). Here, I began to explore whether there are differences in women's biological and subjective responses to acute social stress depending on whether they were taking HCs containing first, second, or third generation progestins. These specific generations of oral HC were chosen for inclusion in the current study based upon previous work, which reports the largest differences in cortisol responses to stress between these generations of HCs (Herrera et al., 2019[4]). Additionally, these first three generations of oral HCs are among the most commonly prescribed HCs (Brynhildsen, 2014; Hall & Trussell, 2012). As such, investigating differences in women's biological and subjective responses to stress between users of first, second, and third generation oral HCs will provide information that pertains to a large number of women and is an important first step towards beginning to understand the how

[4] Specifically, the largest differences in this study were observed between women taking second and third generation HCs, with those taking first generation HCs exhibiting stress responses more similar to those taking third generation HCs than second generation HCs.

different types of HC use are related to women's stress responses. I predicted that the women's stress responses would be the most dysregulated in women using first and third generation HCs, compared to women using second generation HCs.

Methods

Participants

One hundred forty-four women were recruited from Texas Christian University and the surrounding community (see Table 2 for participant demographics). Participants included women who are NC and had not used HCs within the last year (n = 72), or who are taking oral HCs (n = 72). All data was collected between August 2021 and March 2022, during the global coronavirus pandemic. Sample size was determined by convenience[5]. All women were scheduled to participate during their luteal phase, between days 20 and 24 of their ovulatory cycle, to control for cycle phase-based differences in women's sex steroid hormones, and because previous research finds the largest differences between NC women and women taking HCs during the luteal phase of women's ovulatory cycles (e.g., Kirschbaum et al., 1999). Women included in the HC group were required to have been using their current oral HC for at least three months and were scheduled at the same time in their cycle as NC women, during the active pill phase of their HC treatment. Additionally, they were required to be taking a first, second, or third generation oral HCs. Before enrolling in the study, women were prescreened and asked the start date of their most recent menstrual cycle, the regularity of their cycle, and the length of their

[5] The target sample size was determined with an *a priori* power analysis conducted utilizing G*Power software (Faul et al., 2009) and effect sizes reported by Herrera and colleagues (2019). In this study, researchers investigated cortisol reactivity to an acute stress manipulation between women taking different generations of HCs. The generation of HC that participants were taking had a moderate (f = 0.32) effect on cortisol reactivity in response to a stressor. Based on this analysis, a sample size of 39-50 women per HC generation group is needed to detect differences in cortisol reactivity to an acute stressor, at a minimum. As such, the target sample size was set include 100 NC women, and a total of 150 women using HCs (n = 50 per HC generation group) for a total sample size of 250 women. Due to global unrest, the target sample size has not been met. As such, some analyses of interest are excluded, while others are reported as exploratory.

typical ovulatory cycle. Women were excluded from participation if they: 1) took medications known to impact inflammation or the stress response, 2) had a chronic medical condition, including endocrine disorders, 3) had a body-mass index (BMI) > 30[6], 4) had an acute illness, 5) were pregnant or breastfeeding, or 6) did not adhere to pre-study procedures (i.e., did not fast, did not abstain from alcohol, anti-inflammatory medications, and vigorous exercise for at least 12 hours before the session). Participants were excluded from data analysis if, during their session, they reported being on a fourth generation HC (n = 3) or if they reported being on a medication that would have disqualified them from participation (n = 21). Participants were compensated with course credit or a $25 gift card for their participation.

Table 2

Descriptive Statistics for Participant Demographics of the Data Analytic Sample (N = 120)

Variable	*M* (*SD*)
Age (18-37)	19.24 (1.97)
BMI (16.78-34.31)	21.98 (3.45)
Hormonal Contraceptive Use	
Naturally cycling (*n* = 65)	
First Generation (*n* = 19)	
Second Generation (*n* = 11)	
Third Generation (*n* = 25)	
Race/Ethnicity	
White: 65.0% (*n* = 78)	
Black/African American: 3.3% (*n* = 4)	
Hispanic: 16.7% (*n* = 20)	
Asian/Pacific Islander: 5.8% (*n* = 7)	
Multiracial/Other: 9.2% (*n* = 11)	

Note. BMI = body-mass index.

[6] Four women with a BMI between 30 and 34.31 were included in data analysis. Patterns of results do not change when controlling for the effects of BMI on cortisol or inflammation levels.

Procedure

Participants were pre-screened prior to scheduling to ensure that they met study inclusion criteria and were scheduled for their laboratory session on the appropriate days of their ovulatory cycle. The evening before their study session, participants were contacted to ensure that they complied with pre-study procedures (i.e., fasting, abstaining from anti-inflammatory medication use). All study sessions began between 7-9am to minimize differences caused by fluctuations in circadian rhythms and to increase compliance with fasting requirements. Upon arriving for their sessions, researchers ensured that participants were free from coronavirus symptoms and fever (i.e., had a temperature below 99.9 degrees Fahrenheit) and had complied with pre-study procedures. If participants had current coronavirus symptoms, a fever, or had not complied with pre-study procedures, they were rescheduled. Due to the global coronavirus pandemic which was prevalent throughout the course of the current study, the following safety precautions were taken to ensure the safety of both researchers and participants during sessions: 1) all participants were run in single participant sessions, 2) all participants wore face masks covering their nose and mouth throughout the course of the study, except when providing saliva samples, which they did alone in a private room, 3) researchers maintained a six foot distance from participants whenever possible throughout the course of the study, 4) researchers wore a face masks covering their nose and mouth throughout the course of the study, except for the researcher conducting the TSST, who wore a clear face shield during the stress manipulation, 5) all researchers and participants had their temperature taken and confirmed they were free from symptoms of illness upon arrival, and 6) all study rooms were disinfected in between all study sessions.

Once the study session began, participants first provided informed consent, answered brief baseline survey measures (including their current mood and stress levels using paper and

pencil), and then had their height, weight, and blood glucose levels measured. Blood glucose measurements were obtained via a standard finger prick method, using commercially available glucose tests strips and glucometers. A trained researcher first sterilized the participant's index finger using an alcohol pad, then used a sterile, single-use lancet to prick the side of the participant's index finger to extract one drop of blood. The blood was then placed on a disposable glucometer test strip, and the glucometer provided a blood glucose reading. All researchers were trained in appropriate biosafety measures, including disposal of sharps and biohazard waste and blood born pathogen precautions. Participants with a blood glucose level above 105[7] were considered pre-diabetic and were thanked and dismissed (or rescheduled if they revealed that they had not fasted).

Next, women provided a 4mL passive drool saliva sample (baseline). Upon collection, saliva samples were centrifuged, and supernatant was stored at -80 degrees until later assayed for cortisol and proinflammatory cytokines IL-1β, IL-6, and TNF-α. Participants were then escorted to a separate room to complete the stress condition of the TSST (see below for a description of this task). Following the stress manipulation, participants indicated their mood and stress levels with paper and pencil on a visual analogue scale before beginning survey measures collected as a part of a larger study investigating the relationships between HC use, stress responses, and desires to eat unhealthy foods and consume alcohol. Fifteen minutes after the stress task, participants provided a second 4mL saliva sample (post-stress) and again indicated their mood and stress levels while providing the saliva sample. Finally, participants completed questionnaires about their history of hormonal contraceptive use and demographic information

[7] Three participants were allowed to complete the in-person session with blood glucose levels higher than 105. Patterns of results do not change when controlling for the effects of blood glucose levels on cortisol or inflammation levels.

before providing a final paper and pencil assessment of their mood and stress levels. At the end of the study, participants were orally debriefed, thanked, and compensated.

Measures

Baseline Measures. Prior to the stress task, participants completed baseline measures to assess their recent behaviors and recent health history, along with current mood and stress levels. Participants first listed any chronic illnesses, medication use, medication they had used within the last 12 hours, recent illnesses, and recent vaccination history. They then reported on the number of hours they slept the night before, how long it had been since they had eaten, the number of alcoholic beverages they had consumed in the last 48 hours, and the number of hours of exercise they had engaged in in the last 48 hours.

Visual Analogue Scales: Subjective Positivity of Mood and Subjective Stress Measures. Prior to the stress task, immediately following the stress task, 15 minutes after the stress task, and about 50 minutes after the stress task (at the end of the study session), participants indicated their current mood and stress levels on a visual analogue scale by responding to the questions, "How would you rate your mood right now?" (endpoints: *Very negative*, *Very positive*), and "How stressed do you feel right now?" (endpoints: *Not at all stressed*, *Extremely stressed*). Participants were instructed to mark a vertical line indicating their current mood and stress levels on a horizontal line ranging from the endpoints listed above for each question. These marks were then measured in centimetres, providing a continuous measure of mood and stress levels between 0-11 centimetres at four timepoints throughout the study session.

Trier Social Stress Task. To induce stress, participants were brought into a conference room where they were seated at the end of a long table and instructed to spend the next ten minutes preparing a five-minute speech about why they deserve their dream job, which would be recorded and sent to an independent review team who would evaluate their speech. After five minutes had passed, the research assistant returned to the conference room and advised that, because we were running a few minutes behind that day, the participant would have to give their speech now. Then, participants delivered their five-minute speech to a research assistant who was trained to refrain from providing positive affirmation while the participant was supposedly being recorded by a video camera. If a participant stopped speaking before five minutes had passed, the research assistant advised the participant that they had time remaining and that they must continue speaking. Next, participants completed a surprise mental arthritic task aloud, where they were asked to count down from 1022 by 13's for five minutes. If the participant made a mistake, the research assistant informed them that their response was incorrect, and that they must start over. Participants were required to continue the mental arithmetic task for the full five minutes.

Following the TSST, participants were asked to indicate how comfortable they were with public speaking and mental math on a 7-point Likert scale (endpoints: 1 = *Not at all comfortable*, 7 = *Extremely comfortable*) as differences in comfort with public speaking and mental math may influence how stressful participants found these tasks. Following this, participants completed demographic and survey measures.

Biological Measures. Upon collection, saliva samples were immediately centrifuged and supernatant was stored at -20 degrees until later thawed, centrifuged, and assayed for the following analytes:

Cortisol. Saliva samples were assayed in duplicate for levels of circulating cortisol using commercially available enzyme-linked immunosorbent assay (ELISA) kits (Salimetrics, Carlsbad, CA, United States) per manufacturer instructions. Plates were read using an ELISA machine at 450nm. The intra-assay coefficient of variation (CV) for these assays was 3.53% and the inter-assay CV was 7.79%.

Cytokines. Saliva samples were also assayed in duplicate for levels of proinflammatory cytokines IL-1β, IL-6, and TNF-α using commercially available multiplexing assay kits (Miso Scale Delivery [MSD], Rockville, MD, United States) per manufacturer instructions[8]. The intra-assay CV for these assays was 4.40% and the inter-assay CV was 4.82%.

Demographic Measures. Participants completed demographic measures assessing age, race, socioeconomic status (SES), smoking status, sexual activity status, and relationship status.

Data Analysis Plan

All data were assessed for normality and outliers prior to analyses. Cortisol levels and inflammatory biomarkers were severely positively skewed and were log transformed[9], per convention (e.g., Genser et al., 2007; Stoffel et al., 2021). See Table 3 for skewness and kurtosis statistics before and after transformations. Seven outliers remained across measures (≥ 3 standard deviations [*SD*s] from the mean). These were truncated to approximate a normal distribution, by replacing these values with values +/-3 *SD*s from the appropriate means.

[8] While the manufacturer instructions recommend a 1:1 dilution factor, we utilized a 2:1 dilution factor to allow us to capture the low inflammation levels typical of healthy college students.

[9] While a square root transformation resulted in a more normal distribution for baseline cortisol levels (Skewness = 0.35, Kurtosis = 0.06) compared to a log transformation, this was not the case for post-stress levels of cortisol (Skewness = 0.92, Kurtosis = 1.96), which more closely approximated a normal distribution when log transformed. In order to allow comparisons between baseline and post-stress levels of cortisol in repeated measures analyses of variance, log transformations were retained for both timepoints of cortisol.

Table 3

Skewness and Kurtosis Statistics for Cortisol and Inflammatory Biomarkers Before and After Transformations

	Raw Values		Log Transformed Values		Log Transformed and Windsorized Values	
	Baseline	**Post-Stress**	**Baseline**	**Post-Stress**	**Baseline**	**Post-Stress**
Skewness						
Cortisol	1.30	2.34	-0.70	-0.41	-0.50	-0.31
IL-1β	3.18	5.35	-0.98	-0.61	-0.95	-0.61
IL-6	4.68	8.03	0.75	0.34	0.75	0.26
TNF-α	9.39	9.09	0.57	0.47	0.27	0.23
Kurtosis						
Cortisol	2.69	7.56	0.97	1.18	0.04	0.84
IL-1β	12.63	38.42	0.94	-0.46	0.78	-0.46
IL-6	23.95	72.62	0.78	1.30	0.78	1.07
TNF-α	95.52	89.86	1.32	1.95	-0.05	1.04

Note. Windsorized values have had outliers trimmed to +/-3 standard deviations from the mean; IL-1β = interleukin one beta; IL-6 = interleukin six; TNF-α = tumor necrosis factor alpha.

First, I investigated differences in subjective stress levels reported throughout the course of the study between NC women and women using HCs by performing a mixed-model 4 (within-subjects time: baseline vs. immediately post-stress vs. post-stress, vs. end of study) X 2 (between-subjects HC use: NC vs. HC) analysis of variance (ANOVA) on subjective, self-reported stress levels, conducted using IBM's SPSS statistical package version 25 (IBM Corp., 2018). Simple effects were investigated utilizing Tukey's Least Significant Difference follow-up tests to probe differences in marginal means. To investigate how women's moods were impacted by stress, I then performed a mixed-model 4 (within-subjects time: baseline vs. immediately post-stress vs. post-stress, vs. end of study) X 2 (between-subjects HC use: NC vs. HC) ANOVA

on subjective, self-reported positivity of mood. Higher subjective mood levels indicate more positive moods, while lower subjective mood levels indicate more negative moods. Next, I investigated differences in cortisol levels and inflammatory biomarkers between NC women and women using HCs using a series of mixed-model 2 (within-subjects time: baseline vs. post-stress) X 2 (between-subjects HC use: NC vs. HC) ANOVAs on cortisol levels and levels of inflammatory biomarkers.

Following these analyses, I computed difference scores for cortisol and inflammatory biomarkers to investigate relationships between changes in these variables before and after stress, by subtracting baseline values from post-stress values for each variable (e.g., Kapuku et al., 2002; Steptoe et al., 2002). I then used these computed change scores to investigate if HC use (dummy coded: NC = 0 vs HC = 1) moderated the relationship between changes in cortisol levels and changes in inflammatory biomarkers. When significant or trending two-way interactions emerged, I utilized simple slope analyses to investigate relationships between changes in levels of cortisol and changes of levels of inflammatory biomarkers following stress in both NC women and women using HCs. Additionally, I utilized regions of significance tests to investigate differences between NC women and women using HCs in changes of inflammatory biomarkers at high and low levels of cortisol change (i.e., +/- one *SD* from the mean).

To investigate if HC use moderated relationships between changes in cortisol levels and changes in subjective stress levels and positivity of moods following stress, I computed a change score for subjective appraisals of both stress levels and mood (computed using timepoints of stress and mood responses which occurred concurrently with saliva collection). Based on results of these analyses, I then explored the possibility that the impact of changes in cortisol levels on

a) changes in subjective stress levels and b) changes in subjective positivity of mood following stress are both a) moderated by HC use and b) mediated through changes in levels of inflammatory biomarkers, by conducting a moderated mediation analysis using SPSS PROCESS macro (version 3.3) Model 7 (a first-stage moderated-mediation) and Model 58 (a dual-stage moderated-mediation). Significance was determined using 5,000 re-iterations of the data and 95% confidence intervals (CIs).

Finally, I performed exploratory analyses specifically conducted within women taking HCs, to determine if women taking different generations of oral HCs display different subjective responses to stress, using a series of mixed model 4 (within-subjects time: baseline vs. immediately post-stress vs. post-stress vs. end of study) X 3 (between-subjects HC generation: first vs. second vs. third generation HC users) mixed model ANOVAs on subjective appraisals of stress and subjective positivity of mood. I then performed additional exploratory analyses to determine if women taking different generations of oral HCs display different biological responses to stress, using a series of mixed model 2 (within-subjects time: baseline vs. post-stress) X 3 (between-subjects HC generation: first vs. second vs. third generation HC users) mixed model ANOVAs on cortisol and inflammatory biomarkers. While results of these analyses are largely nonsignificant, and analyses underpowered, trending simple effects ($ps \leq .150$) were explored to begin to examine how different generations of HCs impact women's cortisol and inflammatory responses to stress. Finally, I report correlations between changes in cortisol levels, changes in inflammatory biomarkers, changes in subjective appraisals of stress levels, and changes in subjective positivity of mood for each generation of HC users to begin to explore how these variables relate to each other for each generation of HC users. However, due to power

constraints, I do not conduct additional moderated regression analyses between HC generation groups.

Results

Subjective Reponses to Stress

See Table 4 for descriptive statistics for subjective responses to stress.

Table 4

Means and (Standard Deviations) of Subjective Responses to Stress

	Baseline	Immediately Post-Stress	Post-Stress	End of Study
Stress Levels				
Naturally Cycling	4.30 (3.14)	5.01 (2.95)	4.08 (2.86)	3.53 (2.86)
Hormonal Contraceptive	5.16 (2.70)	6.02 (2.85)	5.50 (2.75)	4.79 (2.70)
Positivity of Mood				
Naturally Cycling	8.01 (2.15)	6.18 (2.64)	6.69 (2.35)	7.61 (2.29)
Hormonal Contraceptive	7.48 (1.91)	5.94 (1.92)	6.36 (1.98)	7.31 (1.73)

Subjective Stress Levels

A mixed-model 4 (within-subjects time: baseline vs. immediately post-stress vs. post-stress, vs. end of study) X 2 (between-subjects HC use: NC vs. HC) ANOVA was performed on subjective, self-reported stress levels. A significant main effect of time on subjective stress levels emerged, $F(3, 318) = 16.57$, $p \leq .001$, $\eta_p^2 = .14$. Simple effect analyses revealed that participants reported the highest levels of subjective stress immediately following the stress task ($M = 5.51$, $SE = 0.28$) compared to what they reported at all other timepoints, $ps \leq .002$, and participants reported the lowest levels of subjective stress at the end of the study ($M = 4.16$, $SE = 0.27$) compared to what they reported at all other timepoints, $ps \leq .002$. There were no differences in subjective stress levels reported at baseline ($M = 4.73$, $SE = 0.28$) compared to post-stress ($M = 4.79$, $SE = 0.27$), $p = .780$. Additionally, a significant main effect of HC use on subjective stress

levels emerged, $F(1, 106) = 5.21$, $p = .025$, $\eta_p^2 = .05$. Simple effect analyses revealed that women using HCs ($M = 5.37$, $SE = 0.37$) reported higher levels of subjective stress throughout the study compared to NC women ($M = 4.23$, $SE = 0.34$). A significant two-way interaction between time and HC use did not emerge, $p = .486$. However, follow-up simple, simple effect analyses revealed that, while NC women and women using HCs did not differ in subjective stress levels at baseline, $p = .486$, women using HCs reported marginally more subjective stress immediately post-stress, $p = .073$, and significantly more subjective stress post-stress, $p = .010$, and at the end of the study, $p = .022$, compared to NC women. This pattern of results indicates that women using HCs experienced more subjective stress, specifically following the stress task, than did NC women. Additionally, these results serve as an informal manipulation check of the stress task. As women reported increased stress levels following the stress task, this indicates that women indeed found the stress task to be stressful.

Subjective Positivity of Mood

A mixed-model 4 (within-subjects time: baseline vs. immediately post-stress vs. post-stress, vs. end of study) X 2 (between-subjects HC use: NC vs. HC) ANOVA was performed on subjective, self-reported positivity of mood. A significant main effect of time on subjective mood emerged, $F(3, 321) = 37.57$, $p \leq .001$, $\eta_p^2 = .26$. Simple effect analyses revealed that participants reported the most negative subjective moods immediately post-stress ($M = 6.06$, $SE = 0.22$) compared to all other timepoints, $ps \leq .001$. Moods reported at baseline ($M = 7.74$, $SE = 0.20$) and at the end of the study ($M = 7.46$, $SE = 0.20$) did not differ from each other, $p = .152$, and were each significantly more positive than what participants reported post-stress ($M = 6.53$, $SE = 0.21$), $ps \leq .001$. Neither a main effect of HC use nor a significant interaction between time and HC use emerged on women's subjective mood, $ps \geq .320$. This pattern of results indicates that

NC women and women using HCs experienced similar moods throughout the course of the study, despite women using HCs reporting more subjective stress following the stress task compared to NC women.

Biological Responses to Stress

See Table 5 and Figure 1 for descriptive statistics of biological responses to stress.

Table 5

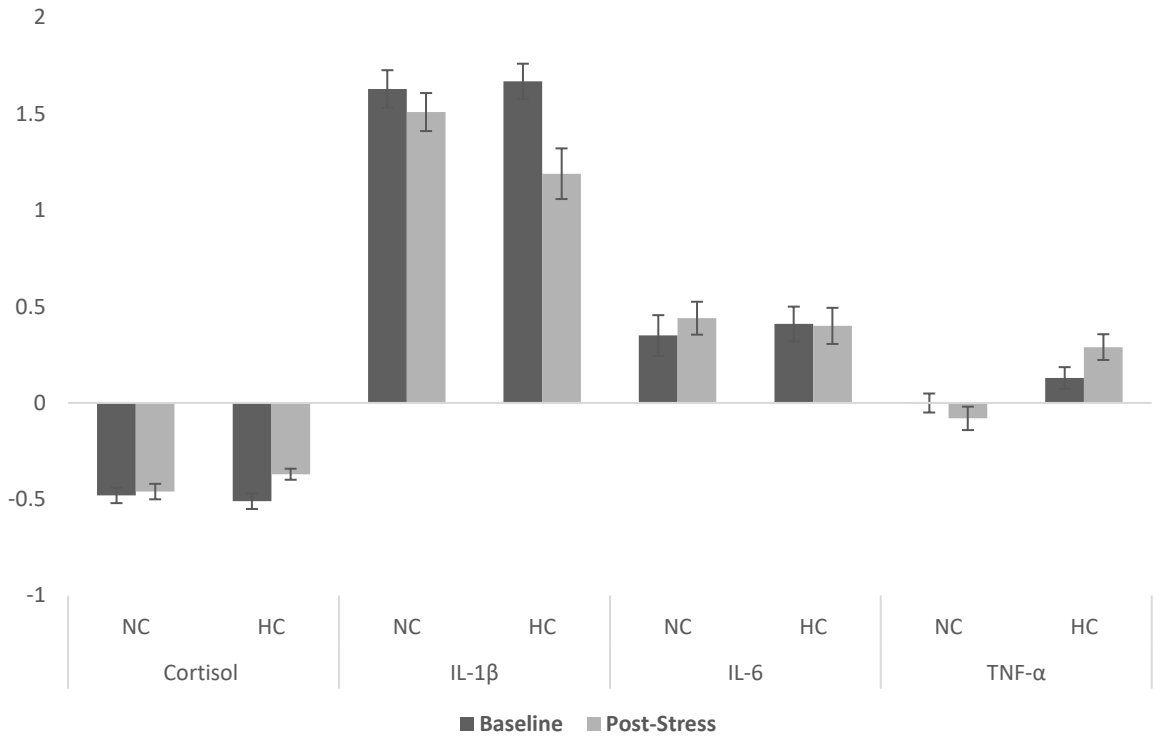
Means and (Standard Deviations) for Cortisol and Inflammatory Biomarkers

	Raw Values		**Transformed Values**	
	Baseline	**Post-Stress**	**Baseline**	**Post-Stress**
NC (*n* = 59-62)				
Cortisol	0.42 (0.28)	0.44 (0.32)	-0.48 (0.31)	-0.46 (0.32)
IL-1β	113.24 (167.96)	85.22 (110.82)	1.63 (0.77)	1.51 (0.76)
IL-6	20.57 (60.31)	17.25 (66.46)	0.35 (0.83)	0.44 (0.66)
TNF-α	2.21 (7.17)	2.39 (4.52)	-0.003 (0.39)	-0.08 (0.47)
HC (*n* = 53-55)				
Cortisol	0.37 (0.23)	0.49 (0.30)	-0.51 (0.30)	-0.37 (0.21)
IL-1β	109.42 (141.25)	87.20 (203.85)	1.67 (0.67)	1.19 (0.97)
IL-6	10.12 (21.52)	7.69 (15.29)	0.41 (0.66)	0.40 (0.69)
TNF-α	2.03 (1.85)	5.27 (17.36)	0.13 (0.41)	0.29 (0.49)

Note. Transformed values have been log transformed, and outliers trimmed to +/-3 standard deviations from the mean; NC = naturally cycling; HC = hormonal contraceptive; IL-1β = interleukin one beta; IL-6 = interleukin six; TNF-α = tumor necrosis factor alpha.

Figure 1

Cortisol and Inflammatory Biomarkers, Reported with Transformed Values



Note. NC = naturally cycling; HC = hormonal contraceptive; IL-1β = interleukin one beta; IL-6 = interleukin six; TNF-α = tumor necrosis factor alpha.

Cortisol Levels

A mixed-model 2 (within-subjects time: baseline vs. post-stress) X 2 (between-subjects HC use: NC vs. HC) ANOVA was performed on cortisol levels. A significant main effect of time emerged, $F(1, 114) = 9.93$, $p = .002$, $\eta_p^2 = .08$. Pairwise comparisons revealed cortisol levels increased from baseline ($M = -0.49$, $SE = 0.03$) following stress ($M = -0.42$, $SE = 0.03$). However, this main effect was qualified by a significant two-way interaction between time and HC use on cortisol levels, $F(1, 114) = 8.70$, $p = .004$, $\eta_p^2 = .07$. Probing this interaction by

examining the effect of HC use on cortisol levels at each of the two timepoints (baseline and post-stress) revealed that, while cortisol levels did not differ between the two groups of women at baseline, $p = .548$, NC women had significantly lower levels of cortisol post-stress compared to women taking HCs, $p = .035$. Probing this interaction by looking at the impact of time on cortisol responses within each group of women (HC and NC) revealed that, although there were no differences between pre- and post-stress levels of cortisol for NC women, $p = .884$, women using HCs exhibited a significant increase in cortisol levels following stress, $p \leq .001$. See Table 5 for descriptive statistics for all biological measures. These results reveal that while NC women did not exhibit a change in cortisol levels in response to stress, women taking HCs did exhibit an increase in cortisol following stress. This result is in contrast to other research, which finds that women using HCs exhibit a blunted cortisol response to acute stress compared to NC women (e.g., Kirschbaum et al., 1999; Lovallo et al., 2019; Nielsen et al., 2013; Roche et al., 2013).

IL-1β Levels

A mixed-model 2 (within-subjects time: baseline vs. post-stress) X 2 (between-subjects HC use: NC vs. HC) ANOVA was performed on IL-1β levels. A significant main effect of time emerged, $F(1, 112) = 16.59$, $p \leq .001$, $\eta_p^2 = .13$. Pairwise comparisons revealed that participants had higher IL-1β levels at baseline ($M = 1.66$, $SE = 0.07$) than they did post-stress ($M = 1.35$, $SE = 0.08$). However, this main effect was qualified by a significant two-way interaction between time and HC use on IL-1β levels, $F(1, 112) = 5.50$, $p = .021$, $\eta_p^2 = .05$. Probing this interaction by examining the effect of HC use on IL-1β levels at each of the two timepoints (baseline and post-stress) revealed that while IL-1β levels at baseline did not differ between the two groups of women, $p = .801$, post-stress, NC women had higher levels of IL-1β compared to women taking HCs, $p = .054$. Examining the effect of time on IL-1β levels separately in each group of women

revealed that there were no differences between baseline and post-stress IL-1β levels for NC women, $p = .221$. However, women using HCs exhibited a significant decrease in IL-1β levels following stress, $p \leq .001$. See Table 5 for descriptive statistics for all biological measures. These results reveal that women using HCs exhibited a decrease in IL-1β levels following stress, while NC women did not.

IL-6 Levels

A mixed-model 2 (within-subjects time: baseline vs. post-stress) X 2 (between-subjects HC use: NC vs. HC) ANOVA was performed on IL-6 levels. No significant effects emerged, *p*s ≥ .268. These results reveal that women did not exhibit significant changes in IL-6 levels following stress, and NC women and women using HCs did not significantly differ from each other in their IL-6 response to stress.

TNF-α Levels

A mixed-model 2 (within-subjects time: baseline vs. post-stress) X 2 (between-subjects HC use: NC vs. HC) ANOVA was performed on TNF-α levels. A significant main effect of time on TNF-α levels emerged, $F(1, 109) = 5.42$, $p = .022$, $\eta_p^2 = .05$. Pairwise comparisons revealed that TNF-α levels were significantly higher post-stress ($M = 0.19$, $SE = 0.05$), compared to at baseline ($M = 0.04$, $SE = 0.04$) for all participants. Additionally, a main effect of HC use on TNF-α levels emerged, $F(1, 109) = 6.87$, $p = .010$, $\eta_p^2 = .06$, revealing that women using HCs exhibited higher levels of TNF-α across both timepoints ($M = 0.21$, $SE = 0.05$) compared to NC women ($M = 0.04$, $SE = 0.05$). A significant two-way interaction between time and HC use did not emerge, $p = .206$. These results reveal that women using HCs exhibited higher TNF-α levels compared to NC women, both before and after stress.

Do Changes in Cortisol Predict Changes in Other Stress Responses?

Creation of Change Scores. After investigating differences in subjective and biological responses to stress between NC women and women using HCs, I then sought to determine if changes in cortisol predicted changes in inflammatory biomarkers and changes in subjective responses to stress. To investigate this, I computed difference scores for cortisol, inflammatory biomarkers, and subjective responses to stress to investigate relationships between changes in these variables before and after stress, by subtracting baseline values from post-stress values for each variable, per convention. See Table 6 for correlations between changes in cortisol and changes in inflammation.

Table 6

Correlations Between Change in Cortisol, Change in Inflammatory Biomarkers, and Change in Subjective Responses to Stress

	ΔIL-1β	**ΔIL-6**	**ΔTNF-α**	**ΔStress**	**ΔMood**
	r = .067	*r* = .272*	*r* = .385**	*r* = .016	*r* = -.056
ΔCortisol	*n* = 114	*n* = 114	*n* = 111	*n* = 109	*n* = 110
		r = -.082	*r* = -.028	*r* = -.012	*r* = -.007
ΔIL-1β		*n* = 114	*n* = 111	*n* = 109	*n* = 110
			r = .209*	*r* = -.076	*r* = -.028
ΔIL-6			*n* = 111	*n* = 109	*n* = 110
				r = .073	*r* = -.076
ΔTNF-α				*n* = 107	*n* = 108
					r = -.225*
ΔStress					*n* = 112

Note. NC = naturally cycling; HC = hormonal contraceptive; IL-1β = interleukin one beta; IL-6 = interleukin six; TNF-α = tumor necrosis factor alpha; ** $p \leq .001$, * $p \leq .05$

Moderated Regression Analyses. I next examined (a) the relationship between changes in cortisol in response to the stressor and changes in levels of each of the three measured inflammatory markers and the changes in each of the two measured subjective responses to stress and (b) whether these relationships were moderated by women's HC use status (dummy coded, NC = 0 vs HC = 1). For each of these analyses, two-way interactions were probed using simple slope analyses to investigate the relationship between changes in cortisol levels and changes in inflammatory biomarkers and subjective responses to stress each in NC women and women using HCs. Additionally, regions of significance tests were used to investigate differences between NC women and women using HCs in changes of inflammatory biomarkers at high and low levels of cortisol change (i.e., +/- one *SD* from the mean). See Table 7 for descriptive statistics of changes scores for each group of women. See Table 8a and Table 8b for correlations between changes in cortisol and changes in inflammatory biomarkers and changes in subjective responses to stress, broken down by HC use status.

Table 7

Means and (Standard Deviations) for Difference Scores

	ΔCortisol	**ΔIL-1β**	**ΔIL-6**	**ΔTNF-α**	**ΔStress**	**ΔMood**
NC	.005 (0.21)	-0.13 (0.64)	0.07 (0.72)	0.10 (0.43)	-0.15 (2.13)	-1.39 (2.36)
HC	0.15 (0.30)	-0.48 (0.94)	-0.01 (0.63)	0.14 (0.63)	0.23 (1.98)	-1.13 (2.26)

Note. NC = naturally cycling; HC = hormonal contraceptive; IL-1β = interleukin one beta; IL-6 = interleukin six; TNF-α = tumor necrosis factor alpha; ** $p \leq .001$, * $p \leq .05$

Table 8a

Correlations Between Changes in Biological and Subjective Responses to Stress (NC)

	Naturally Cycling				
	ΔIL-1β	**ΔIL-6**	**ΔTNF-α**	**ΔStress**	**ΔMood**
ΔCortisol	*r* = -.022 *n* = 60	*r* = .415** *n* = 60	*r* = .120 *n* = 58	*r* = -.005 *n* = 57	*r* = .170 *n* = 58
ΔIL-1β		*r* = .027 *n* = 60	*r* = -.118 *n* = 58	*r* = -.212 *n* = 57	*r* = -.212 *n* = 58
ΔIL-6			*r* = .267* *n* = 58	*r* = -.159 *n* = 57	*r* = .188 *n* = 58
ΔTNF-α				*r* = -.103 *n* = 56	*r* = .071 *n* = 57
ΔStress					*r* = -.168 *n* = 60

Note. NC = naturally cycling; IL-1β = interleukin one beta; IL-6 = interleukin six; TNF-α = tumor necrosis factor alpha; ** $p \leq .001$, * $p \leq .05$.

Table 8b

Correlations Between Changes in Biological and Subjective Responses to Stress (HC)

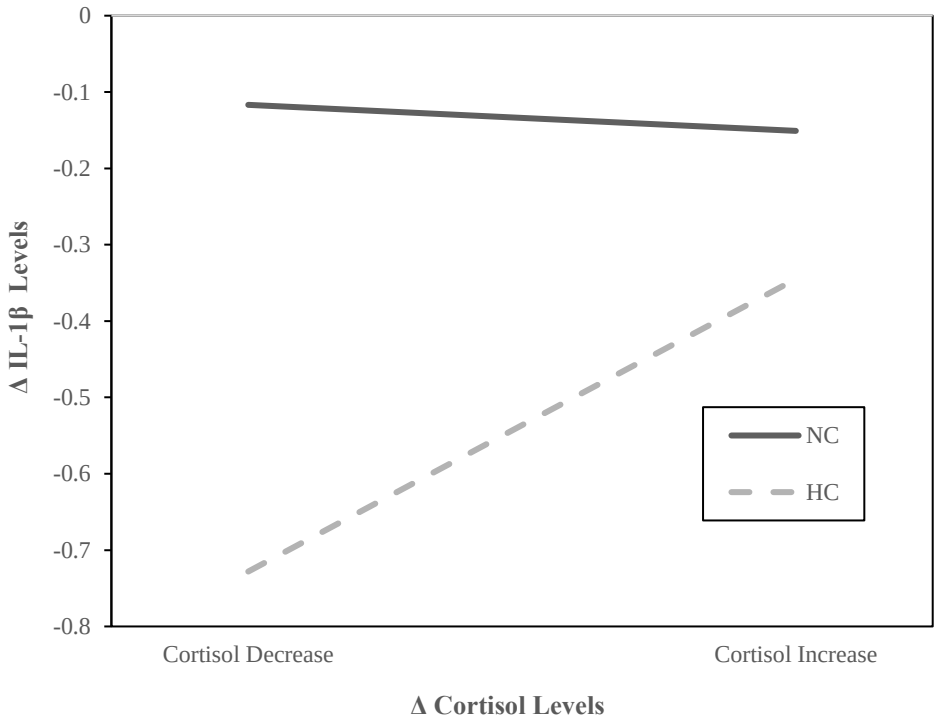
	Hormonal Contraceptive				
	ΔIL-1β	**ΔIL-6**	**ΔTNF-α**	**ΔStress**	**ΔMood**
ΔCortisol	*r* = .225 *n* = 54	*r* = .208 *n* = 54	*r* = .535** *n* = 53	*r* = -.019 *n* = 52	*r* = -.284* *n* = 52
ΔIL-1β		*r* = -.212 *n* = 54	*r* = .029 *n* = 53	*r* = -.007 *n* = 52	*r* = -.103 *n* = 52
ΔIL-6			*r* = .177 *n* = 53	*r* = .043 *n* = 52	*r* = -.178 *n* = 52
ΔTNF-α				*r* = .212 *n* = 51	*r* = -.200 *n* = 51
ΔStress					*r* = -.315* *n* = 52

Note. HC = hormonal contraceptive; IL-1β = interleukin one beta; IL-6 = interleukin six; TNF-α = tumor necrosis factor alpha; ** $p \leq .001$, * $p \leq .05$

Changes in IL-1β Levels. Results revealed a significant main effect of HC use, b = -0.46, SE = .16, t = 2.90, p = .005, such that women using HCs exhibited a larger decrease in IL-1β levels than did NC women. A significant two-way interaction between changes in cortisol levels and HC use on changes in IL-1β levels did not emerge, b = 0.79, SE = .61, t = 1.30, p = .195. See Figure 2 for non-significant interaction effect.

Figure 2

Relationship Between Changes in Cortisol and Changes in IL-1β, Moderated by HC Use



Note. Nonsignificant interaction effect. NC = naturally cycling; HC = hormonal contraceptive; IL-1β = interleukin one beta.

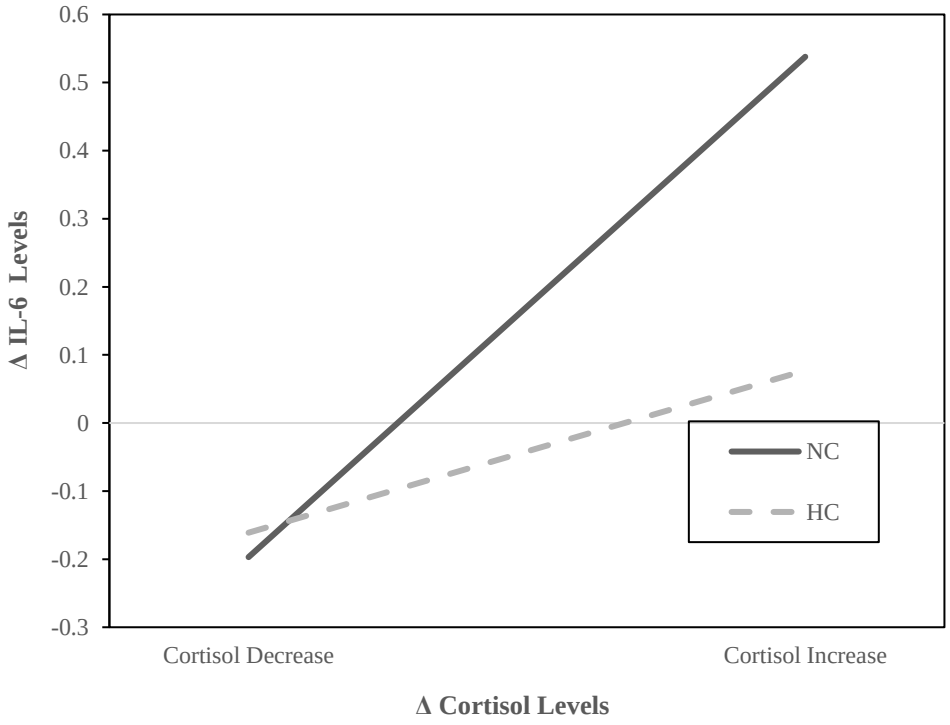
While the interaction between changes in cortisol levels and HC use did not reach statistical significance, simple slope and regions of significance analyses are reported to better understand patterns of inflammatory responses to stress between NC women and women using HCs. Simple slopes analyses revealed a significant positive relationship between changes in cortisol levels and changes in IL-1β levels for women using HCs, $b = 0.72$, $SE = .37$, $t = 1.95$, $p = .054$, and no relationship between changes in cortisol levels and changes in IL-1β levels for NC women, $b = -0.06$, $SE = .48$, $t = 0.14$, $p = .893$. Investigating differences at high and low levels of cortisol change revealed that at low levels of cortisol change (i.e., when cortisol levels decreased after stress) women using HCs had had a larger decrease in IL-1β levels than did NC women, $b = -0.61$, $SE = .22$, $t = 2.83$, $p = .006$, however, at high levels of cortisol change, there were no group differences in the relationship between changes in cortisol and changes IL-1β between NC women and women using HCs, $b = -0.19$, $SE = .23$, $t = 0.85$, $p = .398$. These results reveal that increases in cortisol, while not significantly different between the groups, were associated with a larger decrease in IL-1β levels for women using HCs, but not for NC women.

Changes in IL-6 Levels. Results revealed a marginally significant two-way interaction between changes in cortisol levels and HC use on changes in IL-6 levels, $b = -0.94$, $SE = .49$, $t = 1.90$, $p = .060$. See Figure 3 for interaction effect. Simple slopes analyses revealed a significant positive relationship between changes in cortisol levels and changes in IL-6 levels for NC women, $b = 1.39$, $SE = .39$, $t = 3.57$, $p = .001$, and no relationship between changes in cortisol levels and changes in IL-6 levels for women using HCs, $b = 0.45$, $SE = .30$, $t = 1.49$, $p = .140$. Investigating differences at high and low levels of cortisol change reveal no group differences at low levels of cortisol change (i.e., when cortisol levels decreased after stress) in IL-6 levels, $b = 0.36$, $SE = .18$, $t = 0.20$, $p = .839$, however, at high levels of cortisol change (i.e., when cortisol

levels increased after stress), NC women exhibited a larger increase in IL-6 levels than did women using HCs, b = -0.46, SE = .19, t = 2.47, p = .015. These results reveal that, in NC women, as cortisol levels increased, so did IL-6 levels, while this was not the case for women using HCs.

Figure 3

Relationship Between Changes in Cortisol and Changes in IL-6, Moderated by HC Use

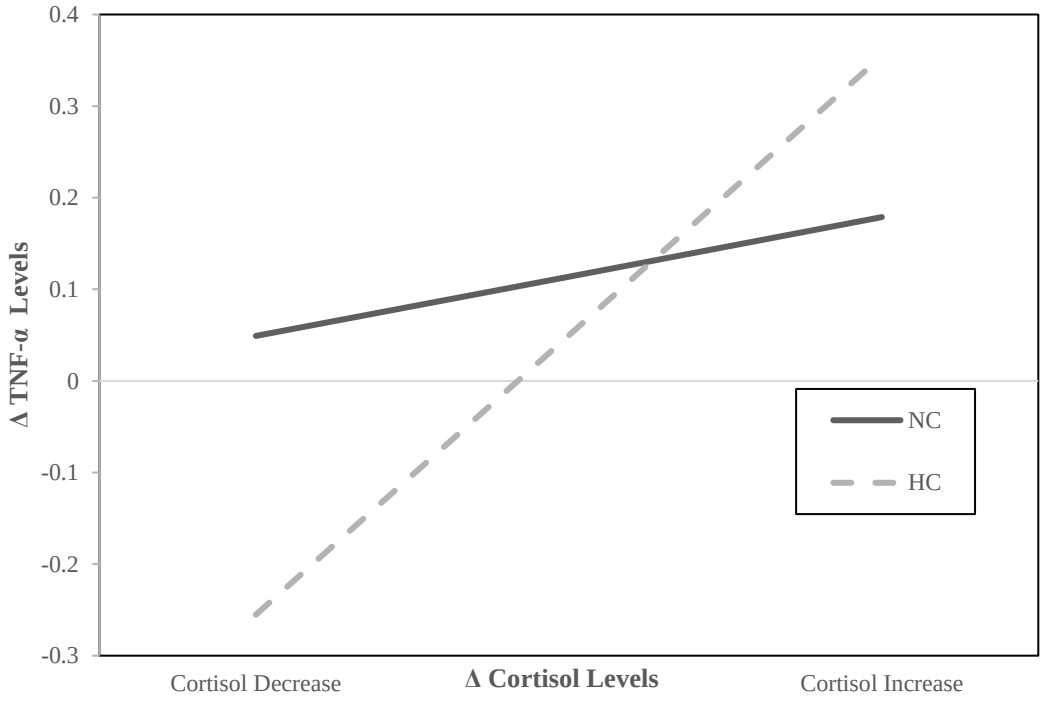


Note. NC = naturally cycling; HC = hormonal contraceptive; IL-6 = interleukin six.

Changes in TNF-α Levels. Results revealed a significant two-way interaction between changes in cortisol levels and HC use on changes in TNF-α levels, b = 0.91, SE = .38, t = 2.37, p = .020. See Figure 4 for interaction effect.

Figure 4

Relationship Between Changes in Cortisol and Changes in TNF-α, Moderated by HC Use



Note. NC = naturally cycling; HC = hormonal contraceptive; TNF-α = tumor necrosis factor alpha.

Simple slopes analyses revealed a significant positive relationship between changes in cortisol levels and changes in TNF-α levels for women using HCs, $b = 1.15$, $SE = .23$, $t = 5.02$, $p \leq .001$, and no relationship between changes in cortisol levels and changes in TNF-α levels for NC women, $b = 0.25$, $SE = .31$, $t = 0.80$, $p = .426$. Investigating differences at high and low levels of cortisol change reveal no group differences at high levels of cortisol change (i.e., when cortisol levels increased after stress) on TNF-α change, $b = 0.18$, $SE = .15$, $t = 1.21$, $p = .229$, however, at low levels of cortisol change (i.e., when cortisol levels decreased after stress),

women using HCs exhibited a decrease in TNF-α levels while NC women did not, b = -0.30, SE = .14, t = 2.25, p = .027. These results reveal that for women using HCs, as cortisol levels increased, so did TNF-α levels, while this was not the case for NC women.

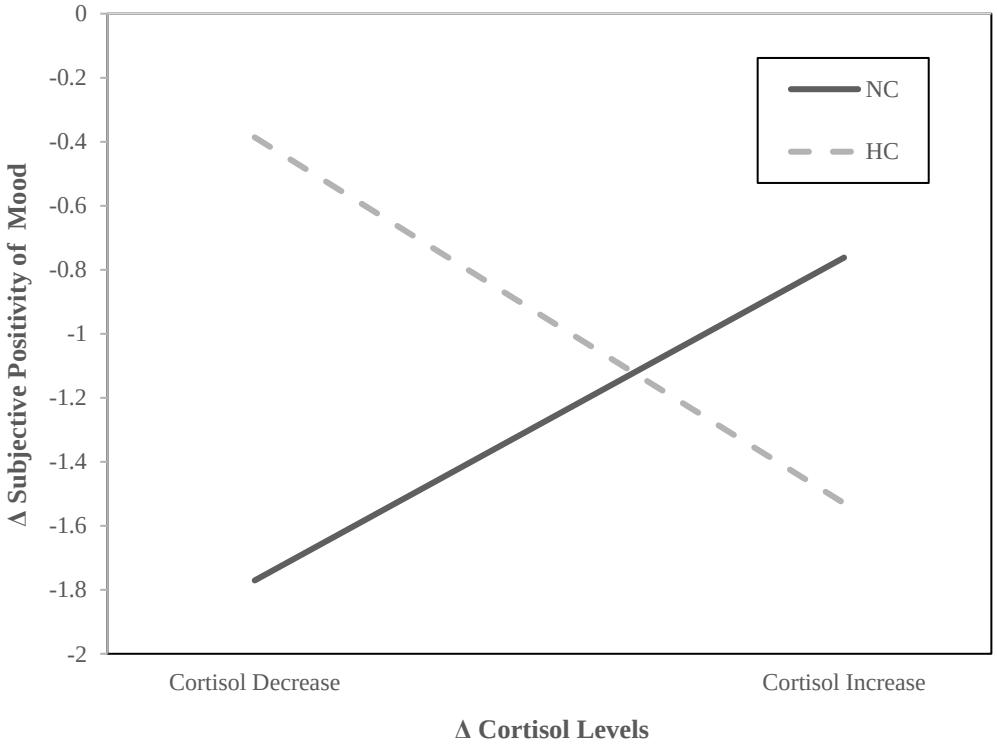
Changes in Subjective Stress Levels. A moderated regression analysis was conducted to investigate if the relationship between change in cortisol levels and change in subjective stress levels was moderated by HC use. Results revealed neither significant main effects of either HC use or change in cortisol levels, nor a significant two-way interaction between changes in cortisol levels and HC use on changes in subjective stress levels, ps ≥ .337. These results reveal that changes in cortisol were not associated with changes in subjective stress levels for either group of women, nor did this relationship differ between NC women and women using HCs.

Changes in Subjective Positivity of Mood. A moderated regression analysis was conducted to investigate if the relationship between change in cortisol levels and change in subjective positivity of mood was moderated by HC use. Results revealed a significant two-way interaction between changes in cortisol and HC use on changes in subjective mood, b = -4.13, SE = 1.82, t = 2.27, p = .025. See Figure 5 for interaction effect. Simple slopes analyses revealed a significant negative relationship between changes in cortisol levels and changes in subjective mood for women using HCs, b = -2.20, SE = 1.09, t = 2.01, p = .047, and no relationship between changes in cortisol levels and changes in subjective mood for NC women, b = 1.94, SE = 1.45, t = 1.33, p = .186. Investigating differences at high and low levels of cortisol change reveal no group differences at high levels of cortisol change (i.e., when cortisol levels increased after stress) on change in subjective mood, b = -0.77, SE = .68, t = 1.13, p = .260, however, at low levels of cortisol change (i.e., when cortisol levels decreased after stress), NC women exhibited a larger decrease in their subjective mood ratings than did women using HCs, b = 1.38,

SE = .63, *t* = 2.18, *p* = .031. These results reveal that changes in cortisol were associated with changes in subjective mood for women using HCs but not for NC women. Here, as cortisol levels increased following stress, women using HCs reported a more negative mood. For NC women, however, increases in cortisol levels following stress were associated with reporting a somewhat less negative mood.

Figure 5

Relationship Between Changes in Cortisol and Changes Subjective Positivity of Mood, Moderated by HC Use



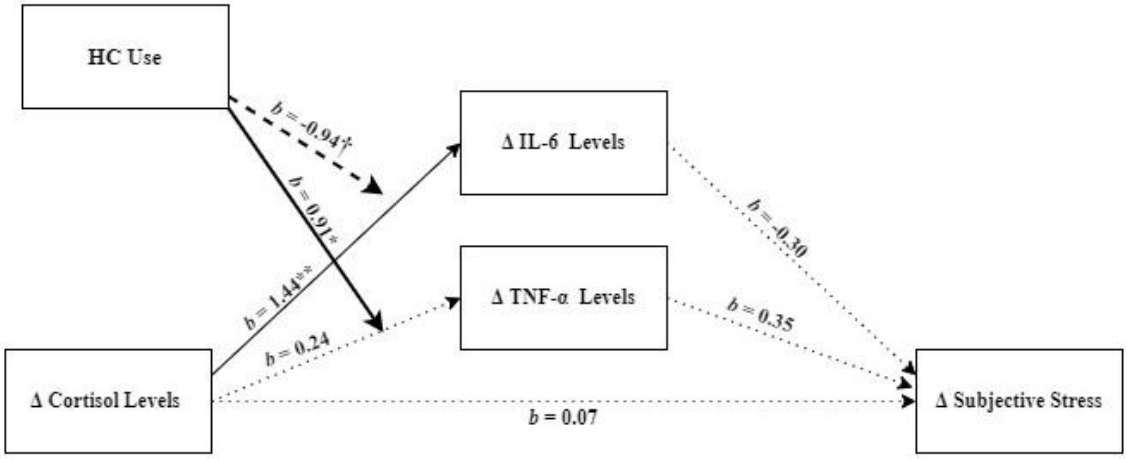
Note. NC = naturally cycling; HC = hormonal contraceptive.

Mediation of Changes in Cortisol on Changes in Subjective Responses to Stress, through Changes in Inflammation, Moderated by HC Use. Previous analyses revealed that HC use moderated relationships between changes in cortisol and changes in both IL-6 and TNF-α levels. Next, I examined if changes in levels of IL-6 and TNF-α mediated relationships between changes in cortisol levels and both changes in subjective stress levels and changes in subjective positivity of mood, moderated by HC use, in both first-stage and dual-stage moderated mediation analyses. Because moderation of relationships between changes in cortisol levels and changes in IL-6 and TNF-α levels have been detailed above, results of these pathways (*a* paths) will not be detailed in the following sections.

Changes in Subjective Stress Levels. A first-stage moderated-mediation analysis was performed to investigate the relationship between changes in cortisol levels and HC use (NC vs. HC) on changes in subjective stress, through changes in IL-6 levels and changes in TNF-α levels (modeled as parallel mediators). SPSS version 23 PROCESS macro version 3.3 model 7 was utilized and significance was determined using 5,000 re-iterations of the data and 95% CIs. See Figure 6a for model with significant paths modeled. Results revealed that changes in cortisol, changes in IL-6, and changes in TNF-α all failed to predict changes in subjective stress levels, *p*s ≥ .338. The indirect effects of changes in cortisol on changes in subjective stress levels were nonsignificant for both NC women and women using HCs, evidenced by 95% CIs which all contained zero. Further, the index of moderated mediation was nonsignificant for both changes in IL-6, 95% CI [-0.41, 1.17], and TNF-α, 95% CI [-0.21, 1.01]. These results indicate that changes in levels of IL-6 and TNF-α do not mediate the relationship between changes in cortisol and changes in subjective stress levels following stress when assessed using a first-stage moderated mediation.

Figure 6a

Mediation of Changes in Cortisol on Changes in Subjective Stress Levels, through Changes in Inflammation, Moderated by HC Use (First-Stage Mediated-Moderation)



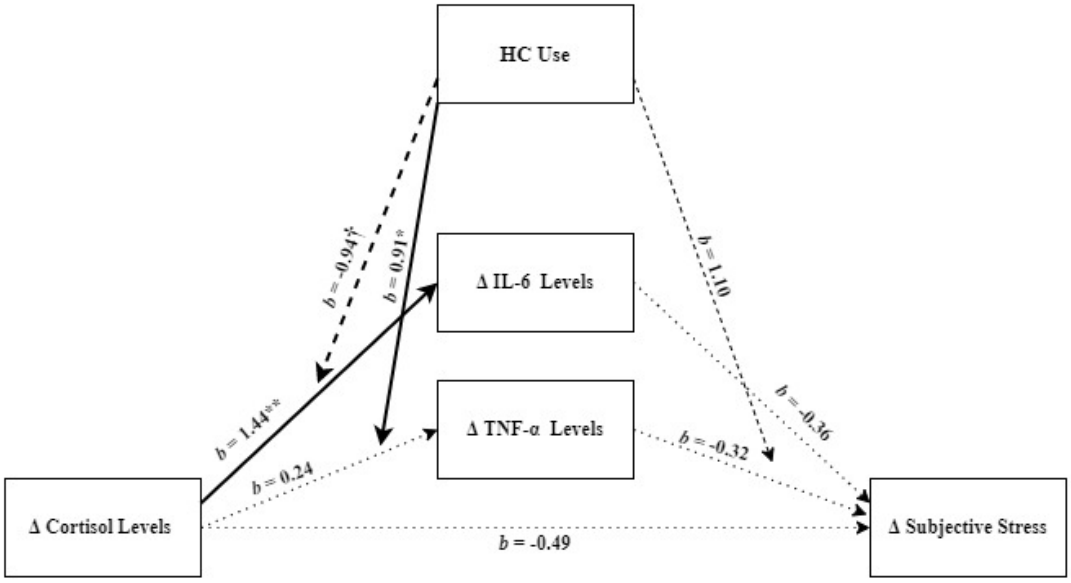
Note. HC = hormonal contraceptive; IL-6 = interleukin six; TNF-α = tumor necrosis factor alpha; ** $p \leq .001$, * $p \leq .05$ † $p \leq .100$.

Next, the same relationships were assessed using a dual-stage mediated-moderation analysis, using PROCESS model 58. See Figure 6b for model with significant paths modeled. In this model, the impact of HC use on changes in subjective stress levels, along with interactions between HC use and both changes in IL-6 and changes in TNF-α levels on changes in subjective stress levels were assessed. While none of these main effects, nor interaction effects reached significance, *p*s ≥ .201, the inclusion of these effects did impact the indirect effects of the model. Specifically, while indirect effects of changes in cortisol levels on changes in subjective stress levels through changes in IL-6 levels remained nonsignificant, as did the indirect effect of changes in cortisol on changes in subjective stress levels through changes in TNF-α levels for

NC women (evidenced by 95% CIs which contained zero), the indirect effect of changes in cortisol on changes in subjective stress levels through changes in TNF-α levels for women using HCs was significant, 95% CI [0.17, 1.69]. Additionally, while the index of moderated mediation for changes in IL-6 levels remained nonsignificant, 95% CI [-1.09, 2.05], the index of moderated mediation for changes in TNF-α levels was significant, 95% CI [0.16, 1.85].

Figure 6b

Mediation of Changes in Cortisol on Changes in Subjective Stress Levels, through Changes in Inflammation, Moderated by HC Use (Dual-Stage Mediated-Moderation)



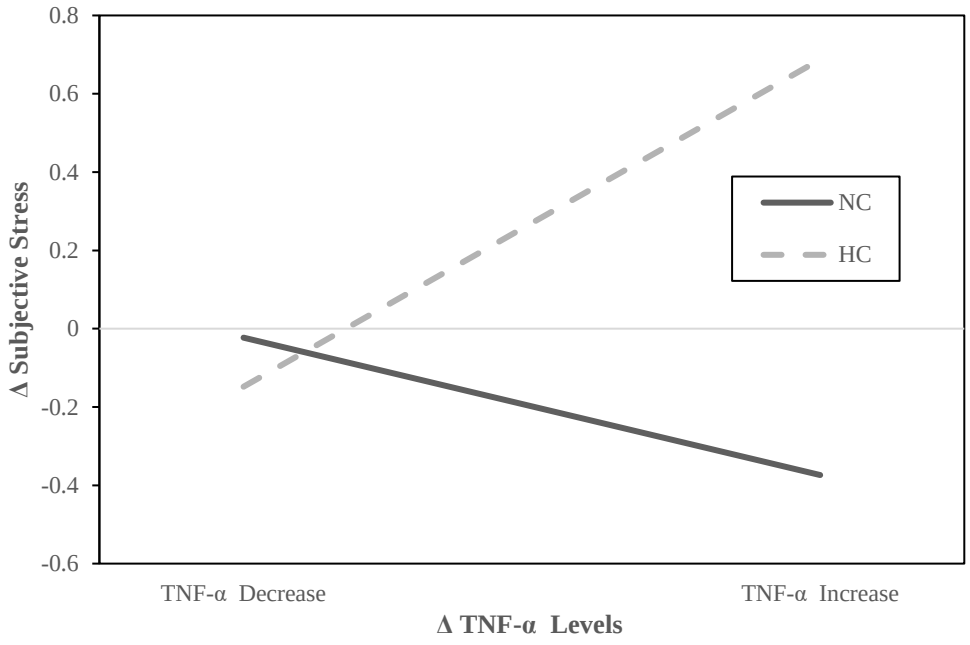
Note. HC = hormonal contraceptive; IL-6 = interleukin six; TNF-α = tumor necrosis factor alpha; ** $p \leq .001$, * $p \leq .05$ † $p \leq .100$.

This result indicates that changes in TNF-α levels mediated the relationship between changes in cortisol levels and changes in subjective stress levels for women taking HCs, but not NC women, when assessing the moderating impact of HC use in a dual-stage moderated-

mediation model. To better understand how the relationship between changes in TNF-α levels and changes in subjective mood are moderated by HC use, see Figure 7 for the nonsignificant interaction effect between HC use and changes in TNF-α levels on changes in subjective stress levels, $b = 1.10$ ($SE = 0.85$), $t = 1.29$, $p = .201$. Overall, these results reveal that changes in cortisol are associated with larger, positive increases in TNF-α in women using HCs compared to NC women. Further, this increase in TNF-α levels is, in turn, associated with an increase in subjective stress levels following stress in women using HCs, but a decrease in subjective stress levels following stress in NC women.

Figure 7

Relationship Between Changes in TNF-α Levels and Change in Subjective Stress Levels, Moderated by HC use

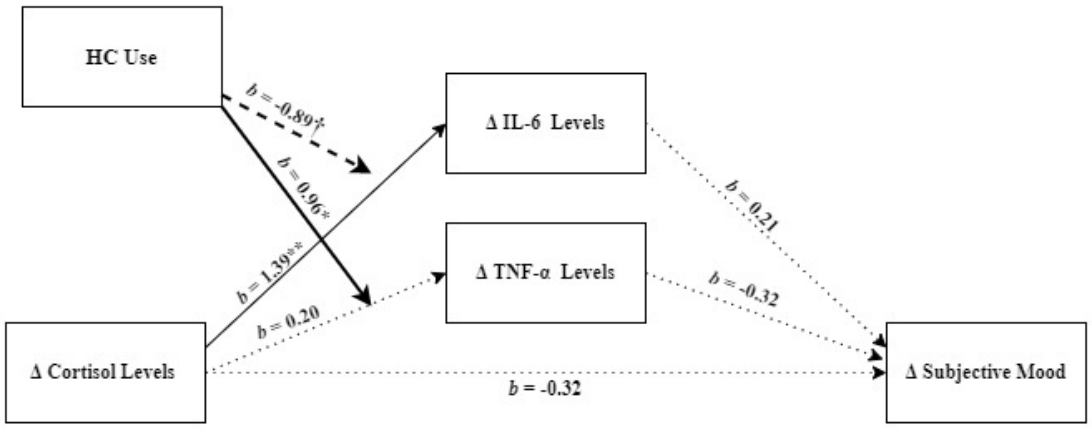


Note. NC = naturally cycling; HC = hormonal contraceptive; TNF-α = tumor necrosis factor alpha.

Changes in Positivity of Subjective Mood. A first-stage moderated-mediation analysis was performed to investigate the relationship between changes in cortisol levels and HC use (NC vs. HC) on changes in subjective mood, through changes in IL-6 levels and changes in TNF-α levels (modeled as parallel mediators). PROCESS macro model 7 was utilized and significance was determined using 5,000 re-iterations of the data and 95% CIs. See Figure 8a for model with significant paths modeled.

Figure 8a

Mediation of Changes in Cortisol on Changes in Subjective Positivity of Mood, through Changes in Inflammation, Moderated by HC Use (First-Stage Mediated-Moderation)

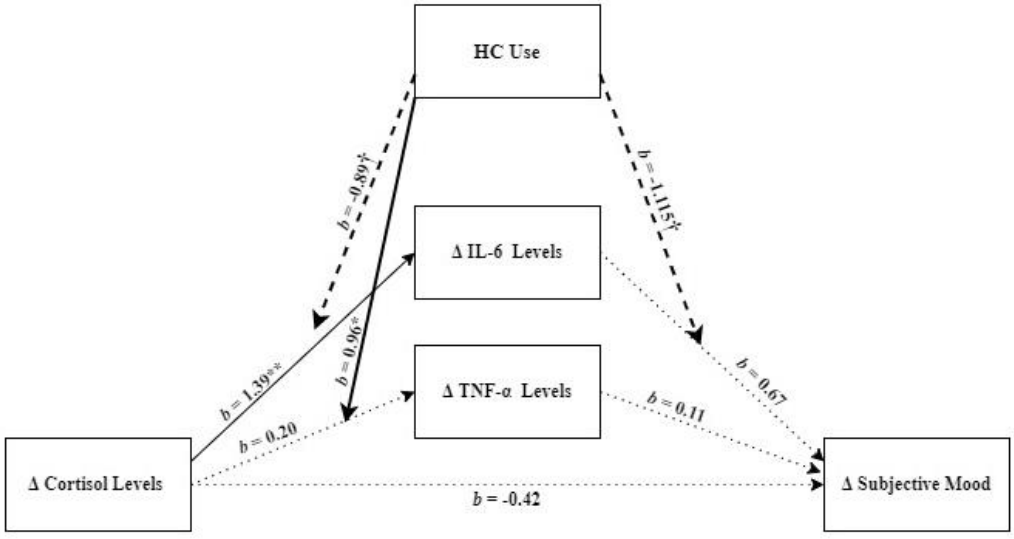


Note. HC = hormonal contraceptive; IL-6 = interleukin six; TNF-α = tumor necrosis factor alpha; ** $p \leq .001$, * $p \leq .05$ † $p \leq .100$.

Results revealed that changes in cortisol, changes in IL-6, and changes in TNF-α all failed to predict changes in subjective mood, *p*s ≥ .480. The indirect effects of changes in cortisol on changes in subjective stress levels were nonsignificant for both NC women and women using HCs, evidenced by 95% CIs which all contained zero. Further, the index of moderated mediation was nonsignificant for both changes in IL-6, 95% CI [-1.50, 0.56], and TNF-α, 95% CI [-1.40, 0.37]. These results indicate that changes in levels of IL-6 and TNF-α do not mediate the relationship between changes in cortisol and changes in subjective positivity of mood following stress when assessed using a first-stage moderated mediation. Next, the same relationships were assessed using a dual-stage mediated-moderation analysis, using PROCESS model 58. See Figure 8b for model with significant paths modeled.

Figure 8b

Mediation of Changes in Cortisol on Changes in Subjective Positivity of Mood, through Changes in Inflammation, Moderated by HC Use (Dual-Stage Mediated-Moderation)

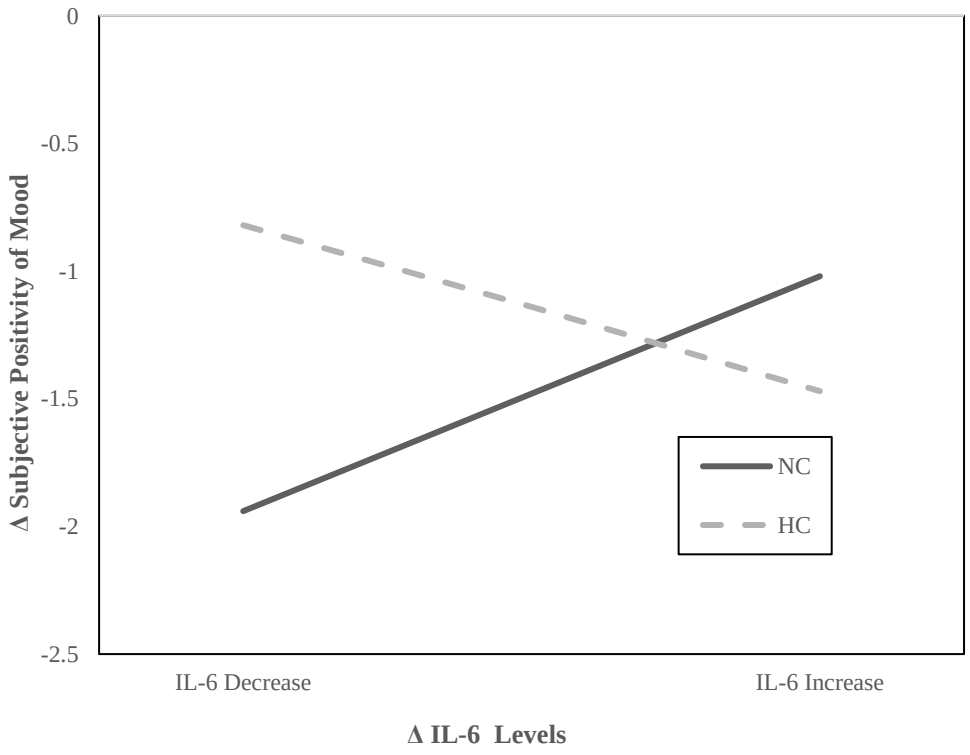


Note. Note. HC = hormonal contraceptive; IL-6 = interleukin six; TNF-α = tumor necrosis factor alpha; ** p ≤ .001, * p ≤ .05 † p ≤ .100.

Here, the impact of HC use on changes in subjective mood, along with interactions between HC use and both changes in IL-6 and changes in TNF-α levels on changes in subjective mood were assessed. While the relationships between HC use, changes in cortisol, changes in IlL-6, and changes in TNF-α on changes in subjective mood were largely nonsignificant, *p*s ≥ .142, one marginally significant two-way interaction emerged between HC use and changes in IL-6 levels on changes in subjective positivity of mood, b = -1.15 (SE = 0.68), t = 1.69, p = .095. See Figure 9 for interaction effect.

Figure 9

Relationship Between Changes in IL-6 Levels and Change in Subjective Positivity of Mood, Moderated by HC use



Note. NC = naturally cycling; HC = hormonal contraceptive; IL-6 = interleukin six.

Simple slope analyses revealed the relationship between changes in IL-6 levels and changes in subjective mood was nonsignificant for both NC women and women using HCs, *p*s ≥ .142. Additionally, at low levels of change in IL-6 (i.e., for those who exhibited a decrease in IL-6 levels following stress) NC women reported a marginally significant larger decrease in the positivity of their moods (i.e., their moods became more negative) compared to women using HCs, b = -0.65 (SE = 0.63), t = 1.79, p = .076, while there were no differences between NC women and women using HCs in the change in the positivity of their moods following stress at high levels of IL-6 change (i.e., for those who exhibited a decrease in IL-6 levels following stress). Despite the emergence of this interaction effect, the inclusion of HC use as a moderator of the relationships between both changes in IL-6 levels and changes in TNF-α levels on changes in subjective stress levels did not impact the indirect effects of the model. The indirect effects of changes in cortisol on changes in subjective stress levels were nonsignificant for both NC women and women using HCs, evidenced by 95% CIs which contained zero. Further, the index of moderated mediation was nonsignificant for both changes in IL-6, 95% CI [-3.69, 0.73], and TNF-α, 95% CI [-2.00, 0.45]. These results indicate that changes in levels of IL-6 and TNF-α do not mediate the relationship between changes in cortisol and changes in subjective positivity of mood following stress when assessed using a first-stage or a dual-stage moderated mediation. However, these results do reveal a pattern, whereby changes in cortisol are associated with larger, positive changes in IL-6 levels in NC women compared to women using HCs. These changes in IL-6, in turn, are positively associated with changes in positivity in subjective mood for NC women, and negatively associated with changes in the positivity in mood for women using HCs. Put differently, everyone reported a more negative mood following stress compared to what they reported at baseline. However, for NC women, increases in IL-6 levels were

associated with a smaller decrement in mood following stress, while, for women using HCs, increases in IL-6 levels were instead associated with larger decrements in mood following stress.

Interim Summary of Results: HC Use

Results have revealed that, compared to NC women, women using HCs reported more subjective stress, and had higher levels of cortisol following stress. Additionally, within women using HCs, increases in cortisol were associated with larger decreases in IL-1β levels, larger increases in TNF-α levels, and a more negative mood following stress. Within NC women, increases in cortisol were associated with increases in IL-6 and a trend towards reporting less negative moods following stress. That is, while all participants reported a more negative mood following stress compared to at baseline, for women using HCs, increases in cortisol were associated with large decrements in mood ratings following stress, while decreases in cortisol were associated with small decrements in mood ratings following stress. For NC women, increases in cortisol were associated with small decrements in mood following stress, while decreases in cortisol were associated with large decrements in mood following stress. Additionally, changes in levels of TNF-α mediated relationships between changes in cortisol and changes in subjective stress levels following stress, specifically for women using HCs. In women using HCs, changes in cortisol were associated with large increases in TNF-α, which were, in turn, associated with reporting increased subjective stress levels following stress, while this was not observed in NC women.

Overall, these results indicate that NC women and women using HCs exhibit different inflammatory responses to stress. Changes in cortisol appear to be more strongly related to changes in IL-6 in NC women than they are in women using HCs, and increases in levels of IL-6 are associated with a somewhat better mood following stress in NC women compared to in

women using HCs. For women using HCs, changes in cortisol appear to be more strongly related to changes in TNF-α than they are for NC women, and increases in levels of TNF-α are associated with heightened appraisals of subjective stress levels following stress for women using HCs, but not for NC women.

Differences Between Generations of HCs

While the current study is not sufficiently powered to investigate differences between women using different generations of HCs in comparison to NC women, I explored differences in subjective and biological responses to stress between women using different generation HCs. First, I performed a set of 4 (within-subjects time: baseline vs. immediately post-stress vs. post-stress vs. end of study) X 3 (between-subjects HC generation: first vs. second vs. third generation HC users) mixed model ANOVAs on subjective stress levels and subjective positivity of mood ratings. Next, I performed a series of mixed model 2 (within-subjects time: baseline vs. post-stress) X 3 (between-subjects HC generation: first vs. second vs. third generation HC users) mixed model ANOVAs on cortisol and inflammatory biomarkers. While results are largely nonsignificant, trending simple effects ($ps \leq .015$) are explored to better understand how use of different generations of HCs relate to women's cortisol and inflammatory responses to stress. Following these analyses, I report correlations between changes in biological and subjective responses to stress for each generation of HC user, to explore patterns in how these variables change together, in women using different generations of HCs.

Subjective Responses to Stress

See Table 9 for descriptive statistics for subjective responses to stress, broken down by HC generation.

Table 9

Means and (Standard Deviations) of Subjective Responses to Stress

	Baseline	Immediately Post-Stress	Post-Stress	End of Study
Stress Levels				
Naturally Cycling	4.30 (3.14)	5.01 (2.95)	4.08 (2.86)	3.53 (2.86)
First Generation	5.60 (2.10)	6.25 (2.39)	5.73 (2.50)	4.96 (2.68)
Second Generation	3.40 (2.88)	4.49 (3.04)	3.74 (3.23)	3.51 (3.02)
Third Generation	5.51 (2.90)	6.46 (3.06)	6.00 (2.58)	4.79 (2.70)
Positivity of Mood				
Naturally Cycling	8.01 (2.15)	6.18 (2.64)	6.69 (2.35)	7.61 (2.29)
First Generation	7.13 (1.53)	5.65 (2.03)	5.93 (2.16)	6.82 (1.75)
Second Generation	7.76 (1.80)	6.86 (2.18)	7.07 (2.39)	7.59 (2.50)
Third Generation	7.66 (2.25)	5.72 (1.67)	6.45 (1.60)	7.62 (1.28)

Subjective Stress Levels. A mixed-model 4 (within-subjects time: baseline vs. immediately post-stress vs. post-stress, vs. end of study) X 3 (between-subjects HC generation: first generation vs. second generation vs. third generation) ANOVA was performed on subjective, self-reported stress levels. A significant main effect of time on subjective stress levels emerged, $F(3, 141) = 6.06$, $p \leq .001$, $\eta_p^2 = .11$. Simple effect analyses revealed that participants reported the highest levels of subjective stress immediately following the stress task ($M = 5.73$, $SE = 0.43$) compared to what they reported at all other timepoints, $ps \leq .013$. Additionally, participants reported higher levels of subjective stress post-stress ($M = 5.16$, $SE = 0.41$) compared to what they reported at the end of the study ($M = 4.54$, $SE = 0.41$). Participants reported the same amount of stress at baseline ($M = 4.84$, $SE = 0.40$) compared to what they reported at all other timepoints, $ps \geq .289$. Additionally, a trending main effect of HC generation on subjective stress levels emerged, $F(2, 47) = 2.35$, $p = .107$, $\eta_p^2 = .09$. Simple effect analyses revealed that second generation HC users ($M = 3.79$, $SE = 0.81$) reported lower levels of subjective stress throughout the study compared to first ($M = 5.63$, $SE = 0.56$) and third

generation (M = 5.79, SE = 0.52) HC users, ps ≤ .066, who did not differ from each other, p = .843.

A significant two-way interaction between time and HC use did not emerge, p = .679. However, follow-up simple effect analyses investigating the impact of HC generation at each timepoint revealed that second generation HC users reported less subjective stress levels than both first and third generation users at baseline and post-stress, ps ≤ .072, and marginally less stress than third generation HC users immediately post-stress, p = .083, but did not differ from first generation HC users at this timepoint, p = .129. First and third generation HC users did not differ from each other at any timepoint, ps ≥ .741, and there were no differences between users of different generations of HCs at the end of the study, ps ≥ .126. Additional follow-up simple effect analyses investigated the impact of time within each HC generation, and revealed that women using first generation HCs reported the lower stress levels at the end of the study compared to immediately post-stress or post-stress, ps ≤ .077. Baseline stress levels for women using first generation HCs did not differ from stress levels reported at any other timepoint, ps ≥ .159, and immediately post-stress subjective stress levels were marginally higher than post-stress stress levels, p = .090. Second generation HC users reported marginally higher stress levels immediately post-stress compared to post-stress, p = .092, but otherwise exhibited no differences in stress levels throughout the study, ps ≥ .146. Finally, women using third generation HCs reported more subjective stress immediately post-stress compared to at baseline and at the end of the study, ps ≤ .056, more stress post-stress than at the end of the study, p = .039, and reported no other differences in subjective stress levels, ps ≥ .245. This pattern of results indicates that women using second generation HCs experienced less subjective stress throughout the course of the study than did women using first or third generation HCs.

Subjective Positivity of Mood. A mixed-model 4 (within-subjects time: baseline vs. immediately post-stress vs. post-stress, vs. end of study) X 3 (between-subjects HC generation: first generation vs. second generation vs. third generation) ANOVA was performed on subjective, self-reported positivity of mood. A significant main effect of time on subjective mood emerged, $F(3, 141) = 13.24$, $p \leq .001$, $\eta_p^2 = .22$. Simple effect analyses revealed that participants reported a more positive mood at baseline ($M = 7.52$, $SE = 0.29$) compared to what they reported immediately post-stress ($M = 6.11$, $SE = 0.29$) and post-stress ($M = 6.48$, $SE = 0.30$), $ps \leq .005$, which did not differ from their reported mood at the end of the study ($M = 7.34$, $SE = 0.26$), $p = .544$. Additionally, participants reported a more negative mood immediately post-stress compared to what they reported at the end of the study, $p \leq .001$, and a marginally more negative mood immediately post-stress compared to what they reported post-stress, $p = .089$. Neither a significant main effect of HC generation nor a significant two-way interaction between time and HC use on subjective mood emerged, $ps \geq .310$.

Follow-up simple effect analyses investigating the impact of HC generation at each timepoint revealed no differences in mood between different generations of HC users at any timepoint. Additional follow-up simple effect analyses investigated the impact of time within each HC generation on subjective mood, and revealed that women using first and third generation HCs reported a more positive mood at baseline and at the end of the study compared to both immediately post-stress and post-stress, $ps \leq .027$, while the positivity of their mood did not differ between baseline and at the end of the study, $p = .469$, nor did it differ between immediately post-stress and post stress, $p = .385$, for first generation HC users. Third generation HC users, on the other hand, reported a more positive mood post-stress compared to what they reported immediately post-stress, $p = .043$. Finally, women using second generation HCs did not

report differences in the positivity of their moods between any timepoints of the study, *p*s ≥ .150. This pattern of results indicates that while women using first and third generation HCs experienced a more negative mood following the stress task, that women using second generation HCs retained a more positive mood throughout the course of the study than did the other generations of HC users.

Biological Responses to Stress

See Table 10 and Figure 10 for descriptive statistics for biological responses to stress, broken down by HC generation.

Table 10

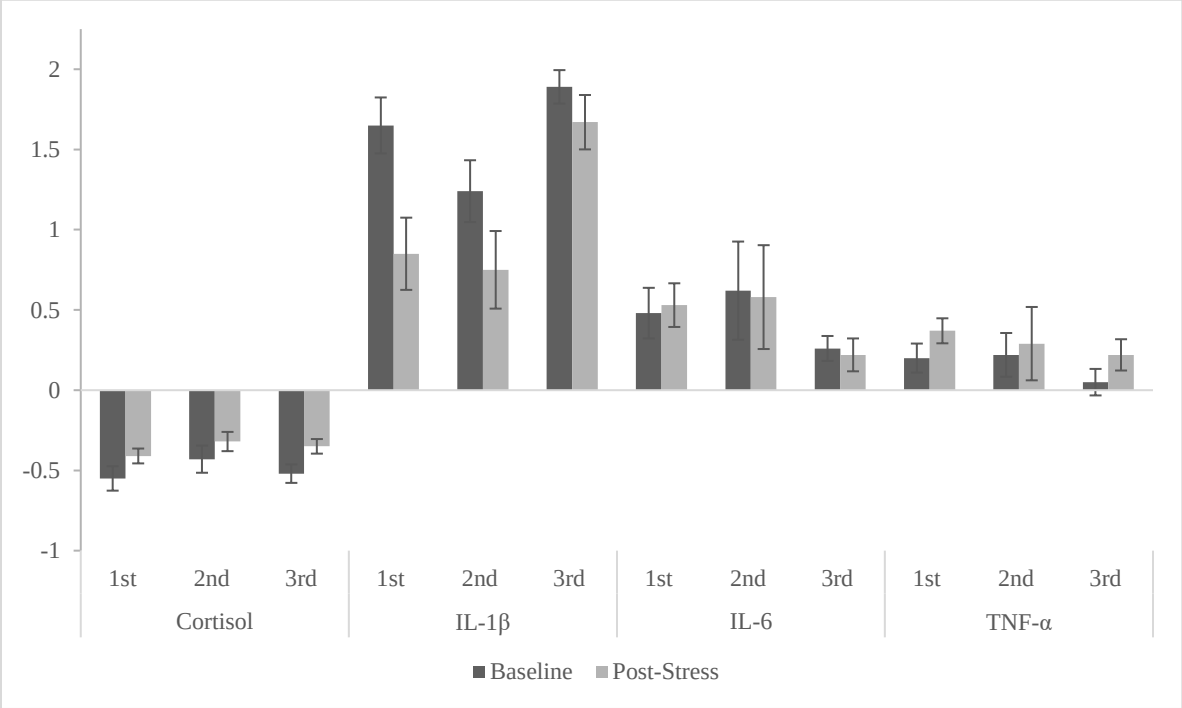
Means and (Standard Deviations) for Cortisol and Inflammatory Biomarkers, Reported by Generation of HC Use

	First Generation HC		**Second Generation HC**		**Third Generation HC**	
	Baseline	**Post-Stress**	**Baseline**	**Post-Stress**	**Baseline**	**Post-Stress**
Cortisol	-0.55 (0.33)	-0.41 (0.20)	-0.43 (0.28)	-0.32 (0.20)	-0.52 (0.29)	-0.35 (0.23)
IL-1β	1.65 (0.76)	0.85 (0.98)	1.24 (0.64)	0.75 (0.80)	1.89 (0.51)	1.67 (0.83)
IL-6	0.48 (0.69)	0.53 (0.59)	0.62 (1.02)	0.58 (1.07)	0.26 (0.38)	0.22 (0.50)
TNF-α	0.20 (0.40)	0.37 (0.34)	0.22 (0.45)	0.29 (0.72)	0.05 (0.41)	0.22 (0.48)
n	19		10-11		24-25	

Transformed values have been log transformed, and outliers trimmed to +/-3 standard deviations from the mean; NC = naturally cycling; HC = hormonal contraceptive; IL-1β = interleukin one beta; IL-6 = interleukin six; TNF-α = tumor necrosis factor alpha.

Figure 10

Means and (Standard Deviations) for Cortisol and Inflammatory Biomarkers, Reported by Generation of HC Use



Note. 1st = first generation HC users; 2nd = second generation HC users; 3rd = third generation HC users; IL-1β = interleukin one beta; IL-6 = interleukin six; TNF-α = tumor necrosis factor alpha.

Cortisol Levels. Results revealed a significant main effect of time on cortisol levels, $F(1, 52) = 9.99$, $p = .003$, $\eta_p^2 = .16$. Pairwise comparisons revealed that women on all generations of HCs experienced a rise in cortisol levels post-stress ($M = -0.50$, $SE = 0.04$) compared to at baseline ($M = -0.36$, $SE = 0.03$). HC generation did not significantly predict cortisol levels, nor was there a significant two-way interaction between time and HC generation on cortisol levels, $ps \geq .452$. Simple effect analyses revealed, however, that first generation HC users experienced a

marginally significant increase in cortisol levels following stress, $p = .057$, and third generation users experienced a significant increase in cortisol levels following stress, $p = .005$, while second generation users exhibited no significant difference between their cortisol levels before and after stress, $p = .294$. No other simple effects comparisons approached significance, $ps \geq .293$. These results are suggestive of a cortisol response to stress being more pronounced in first and third generation HC users than second generation HC users, in contrast to what was found by Herrera and colleagues (2019).

IL-1β Levels. Results revealed a significant main effect of time on IL-1β levels, $F(1, 51) = 14.57$, $p \leq .001$, $\eta_p^2 = .22$. Pairwise comparisons revealed that levels of IL-1β decreased from baseline levels ($M = 1.59$, $SE = 0.09$) following stress ($M = 1.09$, $SE = 0.13$). Additionally, a main effect of HC generation emerged, $F(2, 51) = 7.47$, $p = .001$, $\eta_p^2 = .23$. Simple effect analyses revealed that women using third generation HCs ($M = 1.78$, $SE = 0.13$) had significantly higher levels of IL-1β than did users of both first ($M = 1.25$, $SE = 0.14$) and second generation HCs ($M = 1.00$, $SE = 0.19$), $ps \leq .007$, while first and second generation HC users did not differ from each other in their IL-1β levels, $p = .227$. Additionally, a trending two-way interaction emerged between time and HC generation on IL-1β levels, $F(2, 51) = 2.13$, $p = .129$, $\eta_p^2 = .08$. Simple effect analyses investigating differences between HC generations at each timepoint revealed that, at baseline, second generation HC users had significantly lower levels of IL-1β compared to third generation HC users, $p = .007$, and marginally lower levels of IL-1β compared to first generation HC users, $p = .091$, while users of first and third generation HCs did not differ from each other in IL-1β levels, $p = .227$. Additionally, following stress, third generation HC users had significantly higher levels of IL-1β compared to both first and second generation HC users, $ps \leq .006$, while first and second generation HC users did not differ from each other, $p =$

.772. Simple effect analyses investigating the effect of time within each HC generation revealed that first generation users experienced a significant decrease in IL-1β levels following stress, $p \leq .001$, while second generation users experienced a marginal decrease in IL-1β levels following stress, $p = .085$. Third generation HC users, however, exhibited no differences between their baseline and post-stress levels of IL-1β, $p = .246$. These results suggest that users of third generation HCs experience higher levels of IL-1β that do not decrease in response to stress, in comparison with first and second generation HC users.

IL-6 Levels. Neither of the main effects, the two-way interaction, nor any simple effects approached significance when investigating the impact of time and HC generation on IL-6 levels, $ps \geq .175$.

TNF-α Levels. Neither of the main effects, nor the two-way interaction approached significance when investigating the impact of time and HC generation on TNF-α levels, $ps \geq .210$. However, simple effect analyses revealed that third generation HC users ($M = 0.13$, $SE = 0.06$) exhibited lower levels of TNF-α compared to both first and second generation users ($M = 0.28$, $SEs \leq 0.10$), who did not differ from each other, $p = 1.000$. No other simple effects approached significance, $ps \geq .202$.

Correlations Between Changes in Responses to Stress. To better understand how changes in biological and subjective responses to stress differ between women using different generations of HCs, I explored correlational relationships between changes in these variables. See Table 11 for descriptive statistics of these change scores for each generation of HC users (descriptive statistics for NC women are included for reference). See Tables 12a, 12b, and 12c for correlational relationships between changes in biological and subjective responses to stress for first, second, and third generation HC users, respectively.

Table 11

Means and (Standard Deviations) for Difference Scores, Reported by Generation of HC Use

	ΔCortisol	ΔIL-1β	ΔIL-6	ΔTNF-α	ΔStress	ΔMood
NC	.005 (0.21)	-0.13 (0.64)	0.07 (0.72)	0.10 (0.43)	-0.15 (2.13)	-1.39 (2.36)
1st Gen	0.13 (0.30)	-0.80 (1.09)	0.06 (0.56)	0.17 (0.40)	0.13 (1.65)	-1.20 (1.72)
2nd Gen	0.10 (0.18)	-0.49 (0.67)	-0.04 (0.84)	0.02 (1.09)	0.11 (1.19)	-1.01 (1.95)
3rd Gen	0.18 (0.34)	-0.22 (0.87)	-0.04 (0.60)	0.17 (0.56)	0.37 (2.51)	-1.12 (2.80)

Note. NC = naturally cycling; gen = generation; IL-1β = interleukin one beta; IL-6 = interleukin six; TNF-α = tumor necrosis factor alpha.

Table 12a

Correlations Between Change in Cortisol and Change in Inflammatory Biomarkers, Split by HC Generation: First Generation

	ΔIL-1β	ΔIL-6	ΔTNF-α	ΔStress	ΔMood
ΔCortisol	r = .076 n = 19	r = .592* n = 19	r = .542* n = 19	r = -.118 n = 19	r = -.199 n = 19
ΔIL-1β		r = -.187 n = 19	r = -.366 n = 19	r = -.212 n = 19	r = .237 n = 19
ΔIL-6			r = .522* n = 19	r = -.099 n = 19	r = -.487* n = 19
ΔTNF-α				r = .401† n = 19	r = -.037 n = 19
ΔStress					r = -.194 n = 19

Note. NC = naturally cycling; HC = hormonal contraceptive; IL-1β = interleukin one beta; IL-6 = interleukin six; TNF-α = tumor necrosis factor alpha; ** $p \leq .001$, * $p \leq .05$ † $p \leq .089$.

In first generation HC users, changes in cortisol were significantly positively correlated with changes in IL-6 and changes in TNF-α levels, and changes in IL-6 and changes in TNF-α levels were significantly positively correlated with each other as well. Additionally, changes in

IL-6 levels were significantly negatively correlated with changes in subjective positivity in mood, and changes in TNF-α levels were marginally significantly positively related to changes in subjective stress levels. Overall, when first generation HC users experienced a rise in cortisol, this was accompanied by rises in IL-6 and TNF-α levels, and these rises were accompanied by a more negative mood and heightened appraisals of subjective stress.

Table 12b

Correlations Between Change in Cortisol and Change in Inflammatory Biomarkers, Split by HC Generation: Second Generation

	ΔIL-1β	**ΔIL-6**	**ΔTNF-α**	**ΔStress**	**ΔMood**
ΔCortisol	*r* = -.442 *n* = 11	*r* = -.063 *n* = 11	*r* = .587† *n* = 10	*r* = .035 *n* = 10	*r* = -.423 *n* = 10
ΔIL-1β		*r* = -.725* *n* = 11	*r* = -.260 *n* = 10	*r* = -.163 *n* = 10	*r* = -.147 *n* = 10
ΔIL-6			*r* = .181 *n* = 10	*r* = .411 *n* = 10	*r* = -.005 *n* = 10
ΔTNF-α				*r* = .449 *n* = 9	*r* = -.003 *n* = 9
ΔStress					*r* = -.223 *n* = 10

Note. NC = naturally cycling; HC = hormonal contraceptive; IL-1β = interleukin one beta; IL-6 = interleukin six; TNF-α = tumor necrosis factor alpha; ** $p \leq .001$, * $p \leq .05$ † $p \leq .089$.

In second generation HC users, changes in cortisol level following stress were marginally significantly positively correlated with changes in TNF-α levels. Additionally, changes in IL-1β levels were significantly negatively correlated with changes in IL-6 levels. These results highlight that when second generation HC users experienced a rise in cortisol in response to

stress, this rise was accompanied by a rise in TNF-α levels. Additionally, when they experienced a decrease in IL-1β levels, this decrease was accompanied by a rise in IL-6 levels.

Table 12c

Correlations Between Change in Cortisol and Change in Inflammatory Biomarkers, Split by HC Generation: Third Generation

	ΔIL-1β	**ΔIL-6**	**ΔTNF-α**	**ΔStress**	**ΔMood**
ΔCortisol	*r* = .450* *n* = 24	*r* = .085 *n* = 24	*r* = .677** *n* = 24	*r* = .014 *n* = 23	*r* = -.311 *n* = 23
ΔIL-1β		*r* = .010 *n* = 24	*r* = .490* *n* = 24	*r* = .124 *n* = 23	*r* = -.398† *n* = 23
ΔIL-6			*r* = .004 *n* = 24	*r* = .022 *n* = 23	*r* = -.120 *n* = 23
ΔTNF-α				*r* = .120 *n* = 23	*r* = -.397† *n* = 23
ΔStress					*r* = -.560* *n* = 23

Note. NC = naturally cycling; HC = hormonal contraceptive; IL-1β = interleukin one beta; IL-6 = interleukin six; TNF-α = tumor necrosis factor alpha; ** $p \leq .001$, * $p \leq .05$ † $p \leq .089$.

In third generation HC users, changes in cortisol levels following stress were significantly positively correlated with changes in IL-1β and changes in TNF-α, and changes in IL-1β and changes in TNF-α were also significantly positively correlated with each other as well. Additionally, changes in IL-1β and changes in TNF-α were both marginally significantly negatively correlated with changes in subjective positivity of mood, while changes in subjective stress levels were significantly negatively correlated with changes in subjective positivity of mood. Overall, when third generation HC users experienced a rise in cortisol levels in response to stress, this rise was accompanied by rises in both IL-1β levels and TNF-α levels, which were

accompanied by a more negative mood. A more negative mood was also accompanied by increased appraisals of subjective stress.

Interim Summary of Results: Differences Between HC Generations

Results revealed that, compared to users of other HC generations, second generation users exhibited a more positive mood, and less subjective stress following the stress task. Additionally, compared to users of other HC generations, third generation HC users had a larger cortisol response to stress, heighted IL-1β levels that did not decrease in response to stress, and low levels of TNF-α.

In users of all three generations of HCs, rises in cortisol were accompanied by rises in TNF-α. In first generation HC users, rises in cortisol were also accompanied by rises in IL-6, while in third generation HC users, rises in cortisol were instead accompanied by rises in IL-1β. These rises in levels of proinflammatory cytokines, for first and third generation HC users, were also accompanied by a more negative mood and increased appraisals of subjective stress.

Discussion

For more than three decades now, researchers have reported that women using HCs exhibit a blunted cortisol response to acute social stress compared to men and NC women (Kirschbaum et al., 1999; Lovallo et al., 2019; Nielsen et al., 2013; Roche et al., 2013). Here, I aimed to replicate this effect, and to explore the impact of this dysregulated stress response on women's inflammatory and subjective responses to acute stress. Contrary to my predictions and past research, I found that women using HCs exhibited a more robust cortisol response to stress compared to those who were NC. Additionally, I found that inflammatory responses to stress differed between NC women and women using HCs, with changes in cortisol being positively related to changes in IL-6 in NC women, and changes in cortisol being positively related to TNF-

α in women using HCs. Additionally, these differences in biological responses to stress were associated with differences in subjective responses to stress as well. Increases in cortisol and IL-6 were associated with a more positive mood following stress in NC women compared to women using HCs. For women using HCs, increases in TNF-α levels mediated the relationship between increases in cortisol and increases in subjective stress ratings following stress. Finally, I explored differences in biological and subjective responses to stress between women using first, second, and third generation oral HCs, to begin to understand associations between different types of HC use and women's stress responses.

Subjective Responses to Acute Stress

Overall, women reported a more negative mood following stress compared to their self-reported mood at baseline and reported more subjective stress following the stress task compared to their subjective stress levels reported at baseline, indicating that women found the stress manipulation to be stressful. Additionally, women using HCs reported more subjective stress following the stress task than did NC women, which could indicate that their biological response to stress was less effective at reducing their arousal throughout the course of the study compared to NC women's biological response to stress. While one explanation for this heightened subjective stress response reported by women using HCs could be that they simply found the task to be more stressful, this result could also indicate that women using HCs experience increased anxiety compared to NC women following stress as a result of their HC use. Alternatively, these results could indicate that anxiolytic properties of progesterone – which is typically high in NC women in the luteal phase of their cycles and low in women using HCs – might influence women's subjective appraisals of stress. While NC women and women using HCs did not report differences in the positivity of their moods following stress, results did reveal

differences between NC women and women using HCs in how their biological responses to stress influenced their subjective stress levels and subjective positivity of mood following stress, which I will discuss following discussions of results concerning women's biological responses to stress.

Cortisol Responses to Acute Stress

Previous research has compared stress reactivity between men, women using HCs, and NC women in both the luteal and follicular phase of their ovulatory cycles. Generally, this research finds that men and NC women in the luteal phase of their ovulatory cycles have a larger cortisol response to stress than do women using HCs and NC women in the follicular phase of their cycles (Kirschbaum et al., 1999; Lovallo et al., 2019), especially when tested in morning compared to afternoon sessions (Lovallo et al., 2019; Roche et al., 2013; Sharma et al., 2020), as was done in the current study[10]. However, in the current study, I found women using HCs to exhibit a more robust response to acute stress compared to NC women. While unexpected, others have also failed to find women using HCs to exhibit a blunted cortisol response to stress compared to NC women (e.g., Sharma et al., 2020). In this study, researchers only found blunted cortisol reactivity to stress in women who began using HCs in adolescence compared to those who began using HCs in adulthood, indicating that age at onset of HC use might be an important factor to consider when investigating relationships between HC use and cortisol reactivity to stress, as HPA axis dysregulation reported in HC users in previous research may be driven specifically by women who began HC use during pubertal development.

[10] Additionally, a recent meta-analysis investigating the association between HC use and cortisol reactivity when tested in the afternoon reports robust differences between women using HCs and NC women in the luteal phase when tested in the afternoon (Lui et al., 2016), indicating that this effect is not limited to morning testing sessions, which are more likely to be impacted by diurnal cortisol slopes than are afternoon sessions.

As women using HCs in the current study reported more subjective stress than did NC women following the stress task, women using HCs may have experienced more stress compared to NC women, which may have contributed to their larger cortisol response. However, while the NC women in the current study exhibited a smaller cortisol response to stress than did the women using HCs, this stress response was by no means large in magnitude compared to what has been observed in previous work. Indeed, compared to what has been found in previous studies, most NC women in the current study, and most women using HCs, exhibited a blunted cortisol response to stress.

One potential explanation for the disparity between the results of the current study and results of previous work which finds that women using HCs exhibit a blunted cortisol response to stress compared to NC women is that the current study was conducted during a global pandemic, during which participants may have experienced chronic stress due to increased social isolation, increased environmental pathogen density, increased environmental mortality rates, and increased environmental unpredictability. As many people experienced high levels of ongoing, pandemic-related stress during this time (Ayers et al., 2022; Charles et al., 2021; Kowal et al., 2020), and chronic stress exposure has been found to blunt cortisol reactivity to acute stressors (Lam et al., 2019), potentially through adaptation of stress response systems (e.g., Coffman, 2020), it could be the case that chronic, pandemic-related stress exposure was responsible for the blunted cortisol responses to the acute stress task observed in NC women in the current study. As some have suggested that the HPA axis dysregulation typically observed in women using HCs is akin to what is observed in those who have experienced chronic stress (Hertel et al., 2017), the current results could suggest that under conditions of chronic stress, women using HCs display a more robust cortisol response to stress than do NC women. Future

work should examine this possibility, potentially using non-human animal models, which would allow researchers to experimentally manipulate both HC use and chronic stress exposure. Additionally, it could also be the case that NC women experienced more stress during the pandemic due to their higher probability of becoming pregnant compared to HC women, as women pregnant during the coronavirus pandemic reported high levels of pandemic-related stress (Preis et al., 2020).

Beyond the impact of chronic stress on HPA axis reactivity, chronic stress has also been found to decrease levels of sex steroid hormones, which research suggests may play a large role in modulating the HPA axis mediated stress response. In early work, Kirschbaum and colleagues (1999) suggested that sex differences in cortisol reactivity to stressors, and differences between NC women in different cycle phases and women using HCs, were likely attributable to differences in estradiol levels, alongside the impact of the synthetic estradiol component found in combined oral HCs on the release of corticosteroid binding globulins, which bind to free cortisol, thereby lowering circulating levels of free cortisol. However, since this study was published, researchers have found mixed results when investigating relationships between cortisol reactivity and estradiol levels. Indeed, more recently, researchers find that levels of sex steroid hormones modulate the cortisol response to stress in a sex-specific manner. Specifically, despite finding sex differences in cortisol reactivity to acute stressors, controlling for levels of estradiol, progesterone, and testosterone resulted in no differences in cortisol reactivity between men and NC women (Juster et al., 2016), with progesterone levels being associated with cortisol levels in men, and testosterone levels being associated with cortisol levels in women. In another study, researchers observed a similar effect, whereby within cortisol responders, a difference in cortisol responses to acute stress between men, NC women in the luteal phase, and women using HCs

was eliminated when baseline levels of estradiol, progesterone, and testosterone were controlled for (Barel et al., 2018). Here, researchers discovered negative associations between changes in cortisol following stress and estrogen and progesterone levels in cortisol non-responders. In cortisol responders, these associations were absent, or, in the case of progesterone levels, trending in the opposite direction. These results highlight the need to better understand how sex steroid hormones, which fluctuate across women's ovulatory cycles and differ between NC women and women using HCs, impact cortisol reactivity. While typically, women in the luteal phase of their cycles exhibit high levels of progesterone and relatively high levels of estradiol compared to women in the early follicular phase of their cycles and women using HCs, if women in the current study were indeed experiencing chronic stress, levels of these hormones may have been lower in NC women in the current study compared to NC women in the luteal phase in previous research, which may have contributed to the blunted cortisol response observed among the NC women in the current study. Future research should assess the relationships between sex steroid hormones and cortisol reactivity to stressors by examining the unique influence of these hormones on stress reactivity as opposed to simply controlling for their effects, however, such an endeavor will require far larger sample sizes than what has been employed in much of the extant research in this area.

Inflammatory Responses to Acute Stress

Beyond cortisol reactivity to stress, I also investigated differences in women's inflammatory responses to acute social stress based on their HC use. I found that NC women exhibited lower levels of TNF-α at both timepoints compared to women using HCs, and women using HCs exhibited a decrease in IL-1β levels following stress that was not observed in NC women. Previous work finds women using HCs to exhibit higher levels of IL-6 following stress

compared to NC women, although this result was driven by levels of IL-6 decreasing in NC women following stress (Rohleder et al., 2003). Here, however, I found changes in IL-6 levels for NC women to be positively associated with both changes in cortisol levels and changes in TNF-α levels, while in women using HCs, changes in cortisol levels were positively associated with changes in TNF-α levels and changes in IL-1β levels (although this latter relationship did not significantly differ between NC women and women using HCs). These results suggests that women using HCs have higher levels of TNF-α when compared to NC women, in general, which might contribute to their heightened levels of CRP (e.g., Divani et al., 2015). Additionally, this pattern of results suggests that changes in cortisol levels, even when small in magnitude, are associated with different inflammatory responses in women using HCs compared to NC women. Specifically, changes in cortisol are more strongly related to changes in IL-6 in NC women, while changes in cortisol are more strongly associated with changes in TNF-α in women taking HCs, although future research will be needed to replicate these results before strong conclusions can be drawn.

The positive relationship found in the current study between changes in cortisol levels and changes in IL-6 levels in NC women does not replicate effects found by Rohleder and colleagues (2003), although it should be noted that this previous work measured serum and not salivary IL-6 levels, as was assessed in the current study. This, and other methodological differences between this previous work and the current study warrant caution when directly comparing results, given that salivary and serum proinflammatory cytokine responses to stress are found to differ substantially in their time course (Slavish et al., 2015).

When investigating inflammatory responses to acute stress, researchers find cortisol and proinflammatory cytokine levels to rise in response to stress, for the magnitude of these

inflammatory and cortisol responses to be inversely related (Kunz-Ebrecht et al., 2003), and for IL-6, TNF-α, and IL-1β responses to stress to be similar to each other (Slavish et al., 2015). These patterns were not consistently observed in the current study. While there is no extant research which investigates the differential responses of IL-6, TNF-α, and IL-1β to stress between NC women and women using HCs, it appears likely that women using HCs exhibit a different inflammatory stress response compared to NC women. Some insight into factors which influence differential cytokine responses to stress can be found in work comparing specific proinflammatory cytokine responses to acute stress between men and women. Here, researchers find that men, in response to stress, exhibited an earlier IL-6 peak than do women (Edwards et al., 2006), who exhibited larger IL-6 and smaller TNF-α responses (Steptoe et al., 2002). As such, one speculative possibility is that the strong association between changes in cortisol levels and changes in IL-6 levels observed in NC women in the current study represents a more "female-typical" inflammatory response to acute social stress, whereas the strong association between changes in cortisol and changes in TNF-α observed in HC women represents a more "male-typical" inflammatory response to acute social stress. If replicated in future work, this could indicate that the androgenicity of HCs might be mechanistically responsible for shifting women's inflammatory responses to acute social stress towards a more male-typical, TNF-α dominated inflammatory response to stress.

Currently, there is a paucity of work investigating differences in women's inflammatory response to stress at different ovulatory cycle phases. Previous work suggests that NC women's circulating and LPS-stimulated levels of proinflammatory cytokines are elevated in the early luteal (days 18-22) compared to the late follicular (days 8-12) phase (Willis et al., 2003), however, endogenous hormone levels were not assessed. Women in the current study were in the

mid-luteal (days 20-24) phase of their ovulatory cycles, and were expected to have high levels of progesterone, and somewhat high levels of estradiol compared to in the follicular phase of their cycles, or women using HCs. While progesterone has some known anti-inflammatory effects (Trzonkowski, 2001), the association between sex-steroid hormones and inflammatory processes are nuanced and pleiotropic (see Gilliver, 2010). Despite this, fluctuating levels of progesterone and estradiol throughout the cycle likely impact NC women's inflammatory responses to stress. For example, because NC women in the luteal phase have higher levels of progesterone compared to both NC women in the follicular phase of their cycles or women using HCs, their inflammatory response to stress might be inhibited by progesterone, which may also play a role in potentiating cortisol responses to stress, further inhibiting inflammatory responses to stress in NC women in the luteal phase. While I am unable to test this hypothesis in the current work, future work investigating women's inflammatory response to acute stress between NC women and women using HCs should include groups of NC women in the follicular and luteal phases of their ovulatory cycles and assess endogenous sex-steroid hormone levels both at baseline and in response to stress, and include a no-stress control group. Especially considering recent work finding that sex-steroid hormone levels moderate cortisol reactivity to acute stress (Barel et al., 2018; Juster et al., 2016), this type of design would provide a more thorough and nuanced understanding how sex steroid hormones and HC use interact to predict women's inflammatory responses to acute stress across the ovulatory cycle.

Relationships Between Biological and Subjective Responses to Acute Stress

Past research reports that NC women in the luteal phase of their cycles exhibit a positive association between peak cortisol levels and subjective stress, while women in the follicular phase of their cycles exhibit a negative association between peak cortisol levels and subjective

stress (Duchesne & Pruessner, 2013). Others find and that cortisol reactivity to stress correlates with subjective stress in NC women in the luteal phase, but not in women using HCs or in men (Raymond et al., 2017). In the current work, I find a positive, although not significant, association between post-stress cortisol levels and ratings of subjective stress in both women using HCs and NC women, all of whom were in the luteal phase of their ovulatory cycles, and no relationship between cortisol reactivity and subjective stress reactivity. However, changes in cortisol were differently associated with changes in mood following stress between NC women and women using HCs. Specifically, women using HCs reported a significantly more negative mood in response to increased levels of cortisol following stress, while the opposite pattern of results was observed in NC women. This result provides partial support for my hypothesis that women using HCs would report a more negative mood following stress compared to NC women, although this occurred through changes in levels of cortisol, and not through changes in proinflammatory cytokine levels. That said, I also find a similar positive association between changes in levels of IL-6 and changes in positivity of subjective mood in NC women, with women using HCs exhibiting the opposite pattern. Generally, elevations in IL-6 are found in response to stress (Slavish et al., 2015). Additionally, while IL-6 is typically referred to as a proinflammatory cytokine, it is known to have both pro- and anti-inflammatory effects (see Del Giudice & Gangestad, 2018 for discussion), with anti-inflammatory IL-6 activities often observed in response to exercise stress (Woods et al., 2009). As such, given the relationship between NC women's elevated levels of IL-6 and elevations in cortisol in response stress, in the absence of elevations of other proinflammatory cytokines (specifically the low levels of TNF-α observed following stress in NC women), this increase in IL-6 may reflect anti-inflammatory activities facilitating downregulation of TNF-α and somatic maintenance in NC women, helping

them to return to a more normal state following stress. That elevations in IL-6 levels instead predicted a more negative mood in women using HCs in response to stress could indicate IL-6 is serving a more proinflammatory function in women using HCs, which may be associated with women using HCs struggling to cope with stressful events.

Additionally, I found that changes in TNF-α levels mediated the impact of changes in cortisol levels on changes in subjective stress levels, specifically for women using HCs. That is, increases in cortisol predicted increases in TNF-α levels more strongly for women using HCs than for NC women, and that these increases in TNF-α levels predicted an increase in subjective stress ratings for women using HCs, but not for NC women. This result indicates that increases in cortisol in women using HCs in response to stress is associated with elevated inflammation, and this elevated inflammation predicted heightened appraisals of subjective stress, again implying that women using HCs might struggle to cope with stressful events. Future work should explore these possibilities, however, before strong conclusions are drawn.

Responses to Acute Stress Between Women Using Different Generations of HCs

Beyond investigating biological and subjective responses to acute stress between NC women and women using HCs, I also explored these responses in women using different generations of HCs. Initial exploratory analyses, while insufficiently powered, revealed that compared to users of other HC generations, second generation HC users reported a more positive mood and less subjective stress in response to the stress task. Additionally, compared to users of other HC generations, third generation HC users had a larger cortisol response to stress, heighted IL-1β levels that did not decrease in response to stress, and low levels of TNF-α. In users of all three generations of HCs, rises in cortisol were accompanied by rises in TNF-α levels. In first generation HC users, rises in cortisol were also accompanied by rises in IL-6, while in third

generation HC users, rises in cortisol were instead accompanied by rises in IL-1β. These rises in levels of proinflammatory cytokines, for first and third generation HC users, were also accompanied by a more negative mood and increased appraisals of subjective stress.

Overall, second generation HC users exhibited less reactivity, both subjectively and biologically, to the stress task compared to users of the other generations of HCs, in contrast to results reported by Herrera and colleagues (2019), in which second generation HC users exhibited more reactivity to stressors compared to first or third generation HC users. However, this reactivity in the previous work was driven by women in the inactive pill phase of their cycle, which might explain these disparate results. One potential explanation for the finding in the current work is that second generations HCs are most androgenic of the first three generations of HCs, which could imply that androgenicity of progestins in HCs influences women's reactivity to stress. Another potential alternative or complementary explanation for this finding is that second generation HCs also typically contain the lowest doses of progestins, which could indicate that HCs impact reactivity to stress in a dose dependent fashion. However, as the sample size for this group was smaller than that of the other groups of HC users, these results should be interpreted with some caution.

Additionally, users of third generation HCs displayed a larger cortisol response to stress compared to users of other generations, and heightened IL-1β levels following stress. As third generation HCs are the least androgenic of the first three generations of HCs, these results, again, could suggest that differences in androgenicity are mechanistically responsible for differences in women's responses to acute stress between users of different generations of HCs.

In general, the extant literature investigating differences between users of different types of HCs is limited to few studies conducted with small sample sizes, preventing researchers from

investigating these differences appropriately. Large, well-controlled studies are needed here, to allow researchers the power to investigate outcomes associated with different types of HC use, including differences based on mode of administration, dosage of synthetic estradiol, dosage of progestins, type of progestin, generation of progestin, androgenicity, progestinicity, and estrogenicity of progestins, alongside of individual differences in women, such as duration of HC use, age of HC use onset, endogenous sex steroid hormone levels, chronic stress exposure, and exposure to early-life stress, all of which likely interact to predict differential outcomes for women using HCs, both in relation to their stress reactivity, their moods, and other potential unintended consequences associated with HC use (see Hill & Mengelkoch, 2022, for discussion).

Strengths, Limitations, and Future Directions

While I did not find women using HCs to exhibit a blunted cortisol response to acute social stress, there are a few limitations of the current work which may have contributed to this unexpected result. First, I tested all women before and after acute social stress, without utilizing a no-stress control group. As such, diurnal changes in cortisol levels throughout the course of the study may have resulted in the appearance of a lack of a cortisol response to stress, which would have been detectable if a no-stress control group was included in the current design. Additionally, the study was conducted in the midst of a global pandemic. Beyond the potential impact of experiencing chronic stress on women's HPA-axis reactivity discussed above, conducting the current study during a global pandemic necessitated minor changes to the TSST protocol. Specifically, compliance with social distancing and masking requirements, enacted to ensure the safety of participants and researchers, may have decreased the intensity of the stressor experienced during the TSST; although, given the rise in self-reported, subjective stress reported by all participants following the stress task, it is unlikely that these procedural modifications

played a large role in the current results. An additional limitation to the current work is the reliance on limited timepoints of biological sample collection. Specifically, I collected saliva samples for cortisol and inflammatory biomarkers at two timepoints, once at baseline, and once thirty minutes after the onset of the stress task. It is possible that levels of biological variables may have risen immediately in response to the stress task, which lasted 15 minutes, and had decreased by the time I collected our post-stress saliva sample (30 minutes after the onset of the stressor). Future research would benefit from collecting salvia samples at baseline, immediately following the stress task, 30 minutes after the onset of stress, and about 45 minutes after the onset of stress, to capture the full rise and fall of cortisol and proinflammatory cytokine levels in response to stress. In fact, some researchers recommend collecting saliva samples for up to two hours following the onset of acute stress to capture the full rise and fall of proinflammatory cytokines in response to stress (Slavish et al., 2015). Additionally, all participants in the current study arrived at laboratory sessions after overnight fasting to limit variability in blood glucose levels, which can impact cortisol reactivity to stress. However, some previous work finds low blood glucose levels to blunt reactivity to acute stress (Kirschbaum et al., 1997). As such, it could be the case that generally low blood glucose levels in participants contributed to the generally blunted cortisol reactivity to stress observed in the current study.

One strength of the current study is that I included more participants than did many of the previous studies investigating the impact of HCs on women's stress responses, however, it was still somewhat underpowered to detect effects, particularity when investigating the downstream impact of biological responses to stress on mood. Limited sample size further prevented investigation into individual differences between women which are known to influence stress responses, such as early-life exposure to trauma and age at onset of HC use. Additionally, while

one aim of the current work was to compare the inflammatory responses to stress in women using different types of oral HCs, the study was underpowered to detect differences here, specifically in regard to women using second generation HCs. Further, the current work was limited in that it only included women using first through third generation oral HCs, and only investigated differences in effects based upon generation of HC used, given our sample size. Future work would benefit from investigating how the androgenicity of the progestin used in different HCs impacts women's stress responses, along with the dosage of synthetic hormones contained in the HC method, the method of HC administration, and a women's duration of HC use. For example, in one recent study, researchers compared cortisol responses to acute stress between NC women, women using levonorgestrel containing intrauterine devices (IUDs), and women using levonorgestrel containing oral HCs, and found that the cortisol response to acute stress was potentiated in the women using hormonal IUDs compared to both other groups of women (Aleknaviciute et al., 2017), highlighting the importance of further research into how different types of HC use impacts women's HPA-axis reactivity. Beyond laying the groundwork towards a better understanding of how different forms of HCs are associated with women's abilities of cope with stress, health, and overall well-being, this type of research is vital towards understanding the mechanisms by which at least some types of HC use impacts at least some women's stress responses.

Indeed, while there has been quite a bit of research aimed at measuring HPA-axis dysregulation in women using HCs, little of this work has been aimed at investigating the mechanisms by which this effect occurs. While the current study was not designed to investigate these mechanisms, characterizing the relationships between sex steroid hormone levels and biological responses to stress in women is an important next step towards understanding the

mechanisms by which HC use might impact women's HPA axis mediated stress response. Additionally, investigating how different types of HC use might impact women's stress response is another promising pathway towards understanding, mechanistically, how HC use may impact women's cortisol response to stress. For example, if the androgenicity of progestins contained in HCs is responsible for women's blunted cortisol responses to stress when using HCs, researchers should find large differences in the cortisol response to stress between second and fourth generation HC users. If the synthetic estradiol component of combined HCs is driving this effect, researchers should find large differences between combined and progestin only oral HC users. Answering these mechanistic questions will require researchers to collect much larger sample sizes than what has been employed to date, and to overcome hurdles inherent to conducting research that controls for women's cycle phases, HC use, endogenous sex steroid hormone levels, early-life stress exposure, and genetic differences which can all impact women's stress responses. Likely, the use of well-controlled, prospective studies in which women's stress responses are assessed before and after beginning HC treatment will be needed, along with complementary research utilizing non-human animal models, to fully understand how HC use, and different types of HC use, might impact women's cortisol, inflammatory, and subjective responses to acute stress.

Conclusions

Decades of research finds women's HPA axis-mediated stress response is blunted in women using HCs compared to NC women and men (Kirschbaum et al., 1999; Lovallo et al., 2019; Nielsen et al., 2013; Roche et al., 2013). While I did not replicate this result in the current study, I did find that NC women and women using HCs displayed different inflammatory responses to psychosocial stress, which were differently related to their subjective responses to

stress. In NC women, cortisol and IL-6 rose together in response to stress, and these biological responses to stress were accompanied by more positive moods and reductions in subjective stress levels, indicating that their biological responses may have allowed them to psychologically mange the stress they experienced. In women using HCs, cortisol and TNF-α rose together in response to stress, and these biological responses were accompanied by more *negative* moods and *increases* in subjective stress levels, indicating that their biological responses may not have allowed them to psychologically manage the stress they experienced. Further, while my results provided some initial evidence that women's biological responses to stress differed based upon the generation of progestin contained in the HC they were using, more research here is needed to fully understand how different HCs are related to women's stress responses.

As HC use has been found to increase women's risk for depression (Skovlund et al., 2016) and suicide (Skovlund et al., 2018), and most women in the United States use HCs for at least some period of their lives (Daniels & Jones, 2013), understanding how HC use may impact women's abilities to manage the stress they experience is of grave importance. Given robust relationships between inflammation and depression (Capuron & Miller, 2011; Dantzer et al., 2008; Dooley et al., 2018; Miller & Raison, 2016; Raison & Miller, 2011), further research into how HC use impacts women's inflammatory response to stress offers one promising pathway towards improving the mental health outcomes for the millions of women who currently use HCs, and the many more who will use HCs in the future. Properly investigating these effects will require many large, well-controlled studies, and funding mechanisms which could facilitate this research are currently lacking. I hope that the current results will encourage prioritization of funding targeted at understanding the unintended consequences of HC use, including the

potential impact of HC use on women's biological and subjective responses to stress, as this research has the potential to improve mental health outcomes for millions of women.

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VITA

Summer Mengelkoch was born in Phoenix, Arizona on December 3rd, 1992. She is the second daughter of Richard and Gail Mengelkoch. After graduating from St. Francis Senior High School in 2011, she received a Bachelor of Arts degree from the University of Minnesota in 2013, where she majored in Psychology. In 2017 she began pursuing a Doctor of Philosophy degree at Texas Christian University, under the tutelage of Dr. Sarah E. Hill. Here, she also earned her Master of Science in Experimental Psychology in 2020. After receiving her Doctor of Philosophy degree, Summer will begin working as a Postdoctoral Fellow in the Semel Institute for Neuroscience and Human Behavior at the University of California, Los Angeles with Dr. George Slavich. Summer is partnered to Brocke Addison of Fort Worth, Texas. Together, they care for their dog, Scrunchie, who is also of Fort Worth, Texas.

ABSTRACT

DISTRESSED ABOUT THE STRESS RESPONSE: ASSOCIATIONS BETWEEN HORMONAL CONTRACEPTIVE USE, WOMEN'S STRESS RESPONSE, INFLAMMATION, AND MOOD

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Research finds women using hormonal contraceptives (HCs) exhibit a blunted cortisol response to psychosocial stress, which could have detrimental effects on women's mental health. As such, the current research aimed to better understand women's biological and subjective stress responses. Participants included naturally cycling (NC) women (n = 72) and women using first, second, and third generation oral HCs (n = 72), who were all exposed to the stress condition of the Trier Social Stress task. Researchers assessed women's (a) cortisol responses to stress, (b) inflammatory responses to stress, including pro-inflammatory cytokines (interleukin 1 beta [IL-1β], IL-6, and tumor necrosis factor alpha [TNF-α]), and (c) mood following stress. Additionally, researchers explored if these responses to stress differ based upon (d) the generation of HC women use. Results revealed that while women using HCs did not exhibit a blunted cortisol response to stress compared to NC women, women using HCs and NC women exhibited different pattens of proinflammatory cytokine levels following stress, which also differed between users of different generations of HCs. In NC women, cortisol and IL-6 rose together in response to stress, and these biological responses to stress were accompanied by more positive moods and reductions in subjective stress levels. In women using HCs, cortisol and TNF-α rose together in response to stress, and these biological responses were accompanied by more negative moods and increases in subjective stress levels. These results indicate that women using

HCs, compared to NC women, may struggle to psychologically mange the stress they experience, which may have widespread implications for their mental health.