RESEARCH ARTICLE

Effects of Caffeine and Stress on Biomarkers of Cardiovascular Disease in Healthy Men and Women with a Family History of Hypertension

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Abstract

The connection between caffeine and its potentially detrimental effects on blood markers of cardiovascular disease (CVD) are controversial. Most studies have focused on cholesterol as a putative mediator of the caffeine–CVD relationship. Other blood markers such as C-reactive protein (CRP) and fibrinogen have been understudied. We examined the effects of caffeine and psychological stress on these CVD markers in healthy, young men and women with a confirmed family history of hypertension. A total of 52 normotensive, healthy adults (26 men and 26 women) aged 18–29 years (21.4 ± 0.3) participated in a laboratory session to examine stress reactivity following caffeine consumption. All participants had normal cholesterol levels. Blood pressure (BP), heart rate, serum cortisol and CRP and plasma fibrinogen were collected. Men and women administered caffeine displayed an additional increase in systolic BP and cortisol response to the stressor ($p < 0.05$). Stress interacted with caffeine and sex to alter cortisol, fibrinogen and systolic BP but not CRP levels. These results may shed light on sex-specific pathways that associate caffeine with CVD. Copyright © 2013 John Wiley & Sons, Ltd.

Introduction

Understanding how caffeine influences health outcomes and, in particular, cardiovascular disease (CVD) pathogenesis could improve public health outcomes (e.g. Freedman, Park, Abnet, Hollenbeck, & Sinha, 2012). In the United States, approximately 90% of the population has consumed caffeine by age 11 (James, 1997); therefore, even small physiological effects of caffeine may have important negative health consequences in vulnerable individuals. In addition, the availability of highly caffeinated products has grown and now marketed to young people in the form of energy drinks (for review see Reissig, Strain, & Griffiths, 2009). For the majority of caffeine consumers, exposure to this drug continues across the lifespan with about 75% of caffeine consumed in the form of coffee (Chou, 1992). In the United States, of those individuals who drink coffee, the average consumption is three cups per day (James, 1997).

In 2012, CVD continues to be the leading cause of death in the United States for both men and women [American Heart Association (AHA), 2012]. Known CVD risk markers include elevated resting systolic and/or diastolic blood pressure (SBP/DBP), low density lipoproteins (i.e. cholesterol), triglycerides, blood clotting factors (e.g. fibrinogen) and acute-phase protein, C-reactive protein (CRP; Ridker, 2003; Rodrigues & Klein, 2006). The relationship between caffeine consumption and acute elevations in blood pressure (BP) has been well-established, along with vascular mechanisms for this effect (al' Absi et al., 1998; Greenberg & Shapiro, 1987; Hartley, Lovallo, Whitsett, Sung, & Wilson, 2001; Lovallo et al., 1989, 1991). In addition, caffeine and stress appear to have particularly negative effects on BP and cortisol levels for those with a family history of hypertension relative to those with no family history of hypertension (al' Absi et al., 1998; Lovallo et al., 1989).

Most investigations examining the relationship between caffeine use and blood markers of CVD primarily have focused on blood lipids, including total, high-density and low-density lipoproteins (Jossa et al., 1993; Kohlmeier, Mensink, & Kohlmeier, 1991). Thus, the relationship

between additional CVD biomarkers such as fibrinogen and CRP and caffeine consumption has been understudied, particularly with regard to the inclusion of acute stress (Rodrigues & Klein, 2006).

Acute psychological stress may increase plasma fibrinogen levels. For example, Muldoon and colleagues (1995) found that a frustrating cognitive task led to significant increases in plasma viscosity compared with the rest. An earlier review also suggests that fibrin-D (small fibrinogen fibres that have been broken down) increases in response to chronic and acute psychological distress (von Kanel & Dimsdale, 2003). However, no studies have investigated whether or not caffeine modifies the stress effect on fibrinogen levels.

In a cross-sectional study, higher dietary caffeine intake was linked with elevated plasma fibrinogen levels in Japanese-Americans and Japanese in Japan (Miura et al., 2006). At this time, however, only three studies have examined the effects of coffee or caffeine on fibrinogen in the laboratory (Bak & Grobbee, 1990; Happonen, Salonen, & Seppanen, 1987; Naismith, Akinyanju, Szanto, & Yudkin, 1970). Results are inconsistent, with some reports that coffee consumption increases fibrinogen levels (Happonen et al., 1987), whereas others report no increase in response to coffee or caffeine consumption (Bak & Grobbee, 1990; Naismith et al., 1970). Because of great differences in the methodology of these studies, comparisons between these findings are difficult to interpret (e.g. coffee versus caffeine intake).

As with fibrinogen, few studies have examined the combined effects of stress and caffeine on CRP. With regard to stress alone, an acute laboratory stressor increased CRP in healthy men (Hamer et al., 2006b) and special populations, including depressed women (Miller, Rohleder, Stetler, & Kirschbaum, 2005) and men with coronary artery disease (Steptoe et al., 2003). However, several investigators have found no changes to CRP levels in response to a laboratory stressor (Steptoe, Willemsen, Owen, Flower, & Mohamed-Ali, 2001; Dugue, Leppanen, Teppo, Fyhrquist, & Grasbeck, 1993; for review see Steptoe, Hamer & Chida, 2007). More recently, coffee consumption did not affect CRP reactivity to mental stress among men, even following 4 weeks of coffee abstinence (Hamer, Williams, Vuononvirta, Gibson, & Steptoe, 2006a). Despite these negative *in vivo* results, previous *in vitro* research demonstrated that caffeine exposure enhanced CRP production in human liver cells (Ganapathi et al., 1990).

Importantly, none of these studies examining coffee/caffeine and CRP levels have included women. According to the AHA (2012), in every year since 1984, CVD has claimed the lives of more women than men. Furthermore, the gap between male and female deaths has increased dramatically over the years, which further emphasizes the importance of including females in such studies. Therefore, this study examined sex differences in the effects of psychological stress and

caffeine on CRP and fibrinogen—an important gap in the literature.

Women show diminished cortisol stress reactivity compared with men, especially during the late luteal phase of the menstrual cycle (Kirschbaum et al., 1999; Kudielka et al., 1998). This reduced cortisol responsivity may suggest that women also will have a reduced CRP and fibrinogen response to stress compared with men.

The overall goal of this study was to examine the effects of caffeine and stress on BP, cortisol, CRP and fibrinogen, in healthy male and female participants with a family history of hypertension. We predicted that caffeine administration would interact with stress to additionally increase SBP, DBP and cortisol. We hypothesized a similar stress-induced increase in CRP and fibrinogen and that caffeine exposure would enhance this response. In women, we anticipated reduced BP, cortisol, CRP and fibrinogen reactivity relative to men.

Methods

Participants

Healthy men ($N = 26$) and women ($N = 26$), between the ages of 18 and 29 years (21.4 ± 0.3 years), were recruited to participate in a study on the biobehavioral effects of caffeine through the use of flyers posted around the Penn State campus and local community. A trained researcher initially interviewed potential participants over the telephone to review their health history and to determine eligibility for an initial screening lab session, including the need to have a biological parent under current treatment for high BP. Family history of hypertension status was confirmed by a questionnaire completed by the potential participant's biological parent(s) and returned directly to the laboratory in a pre-paid, lab addressed envelope; family history of hypertension was defined as having at least one parent who was (1) diagnosed with hypertension and (2) currently taking or had taken prescription BP regulation medication in the past year (al'Absi et al., 1998; Greenberg & Shapiro, 1987).

Study exclusion criteria included any significant health problems or use of medications that would interfere with interpretation of BP and blood marker data such as diabetes or hypertension, laboratory resting SBP > 140 mmHg or DBP > 90 mmHg, body mass index (kg/m^2) > 30, documented neurological disorder, history of depression and/or anxiety, cognitive or attentional disorders such as attention deficit/hyperactivity disorder, drug or medication use that would interfere with normal hormonal, metabolic and cardiovascular functioning, including oral and injected corticosteroids, psychostimulants, aspirin, antioxidant vitamins or use of tobacco or other nicotine products and daily caffeine consumption < 100 mg (about one 8-oz. cup of coffee) or > 500 mg. Daily caffeine intake assessed

during telephone screening and later confirmed by questionnaire and health screening during the screening lab visit. For female participants, women were excluded if they used hormonal medication, current or recent (<1 year) pregnancy, or current or recently breastfeeding. Using published methods from both our lab and others, women were brought into the laboratory during the late luteal phase of their menstrual cycle as determined by self-reported date of the last menstrual period and menstrual cycle length (Kirschbaum et al., 1999; Walter et al., 2012; Whetzel et al., 2007). The luteal phase also was confirmed through baseline serum samples that were assayed for estradiol ($165.21 + 21.72$ pg/mL) and progesterone ($27.12 + 4.66$ ng/mL). All women included in this study were in the appropriate phase of their menstrual cycle.

Participant characteristics are shown in Table I. A total of 69% of participants were Caucasian ($N = 36$), 14% were African American ($N = 7$), 11% were Asian ($N = 6$), 4% were Hispanic or Latino ($N = 2$), and 2% were self-reported 'other' ($N = 1$). Ethnicity was equally represented among the caffeine treatment groups and men and women [χ^2 s $(1, 52) < 1.45$, p s > 0.05]. All procedures were reviewed and approved by the Pennsylvania State University Institutional Review Board prior to the initiation of the project.

Procedures pre-laboratory screening session

On the basis of the telephone screening, eligible participants were scheduled for a health screening session at the Penn State General Clinical Research Center (GCRC), which included a 12-h fasting cholesterol assessment (Craig, Amin, Russell, & Paradise, 2000). This screening session was not scheduled at a specific phase of the menstrual cycle for women as other investigators report no effect of menstrual cycle variation on lipoprotein levels (e.g. Elhadd et al., 2003). Participants were asked for permission to have letters and a medical questionnaire sent to their biological parent(s) to confirm parental hypertension status (al'Absi et al., 1998; Greenberg & Shapiro, 1987). The subsequent laboratory stress/caffeine test session was scheduled upon confirmation of normal cholesterol levels (total cholesterol <240 mg/dL, high-density lipoprotein ≥40 mg/dL, low-density lipoprotein <130 mg/dL) and confirmed

positive parental hypertension status through receipt of medical health history forms from the parent(s).

Laboratory session: overview

The experimental design was a mixed model. The between-subjects factors were sex (male and female) and drug administration (placebo and caffeine). The within-subjects factor was stress. Specifically, all participants participated in the same protocol, which consisted of a baseline rest period, a challenge period (13.5-min speech challenge and 15-min mental arithmetic task) and a recovery period. BP and heart rate (HR) were collected every 2 min throughout the laboratory session. Blood samples and self-reported rating of stress using a 7-point Likert scale were collected three times during the study: (1) end of baseline period and immediately prior to the stress protocol, (2) 15 min following completion of the stress protocol and (3) 45 min after the stressor ended.

Baseline

Participants abstained from caffeine use 4 h before the laboratory session and were asked to eat a low-fat lunch as a high-fat meal has been found to significantly increase serum CRP levels (Carroll & Schade, 2003). All sessions were conducted in the GCRC, starting at 1300 h, to control for the diurnal rhythm of cortisol across participants (Van Cauter, 1990). Upon arrival to the GCRC, participants provided informed consent, height and weight were measured, and health status was assessed by a nurse practitioner to confirm eligibility, including a pregnancy test for all female participants. Following completion of questionnaires to determine daily caffeine intake and mood, a standard BP cuff (Dinamap Compact Blood Pressure Monitor, Critikon, Tampa, FL) was placed on the participant's dominant arm, and a trained nurse inserted an indwelling catheter in the non-dominant arm using standard antispetic techniques. Participants were allowed to sit quietly for 30 min while resting BP and HR were collected.

Drug administration

At the end of the 30-min baseline period, anhydrous caffeine (3.3 mg/kg) (Spectrum Chemical Corporation, Gardena, CA) mixed with refrigerated white grapefruit juice (Unsweetened White Grapefruit Juice, Giant®

Table I. Baseline characteristics (means ± SEM) by sex and caffeine treatment groups

	Males		Females		
	0 mg/kg($N = 13$)	3.3 mg/kg($N = 13$)	0 mg/kg($N = 13$)	3.3 mg/kg($N = 13$)	F (p)
Age (years)	21.00 ± 0.45	22.07 ± 0.64	20.38 ± 0.27	22.31 ± 0.87	6.25 (0.016) [a]
Body mass index (kg/m^2)	24.15 ± 0.49	25.52 ± 0.60	24.19 ± 0.93	23.14 ± 0.94	NS
Self-reported daily caffeine intake (mg/day)	259.85 ± 22.64	291.23 ± 27.10	270.31 ± 17.80	231.62 ± 24.02	NS

[a]Caffeine group greater than placebo group.

brand, Landover, MD) was administered to half of the participants. Caffeine dosage was calculated on the basis of the participant's body weight obtained at the beginning of the laboratory session. The placebo group received grapefruit juice without the addition of caffeine. The caffeine dosage and use of grapefruit juice has consistently been used in the administration of caffeine in other studies (e.g. Hartley et al., 2000; Lovallo et al., 1989, 1991). Grapefruit juice has been found to have no effect on caffeine's pharmacokinetics and no hemodynamic effects (Maish, Hampton, Whitsett, Shepard, & Lovallo, 1996), and it was used to mask the bitter taste of caffeine. To keep the experimenter blind to the drug condition, each drink was prepared by a nurse who was given a sealed envelope indicating the drug condition. Participants then were asked to rest for 20 min to allow for caffeine absorption prior to the first blood draw and administration of the lab stressor. BP and HR were measured every 2 min during this rest period.

Stressor

Next, all participants underwent a modified Trier Social Stress Task protocol (Kirschbaum, Pirke, & Hellhammer, 1993) that consisted of preparation (10 min) and delivery of a 3.5 min videotaped speech about a personal failure, followed by 12 min of mental serial subtraction task under time pressure with negative feedback (i.e. counting backwards from a four-digit number by 7's and then 13's). This portion of the study was conducted by a different experimenter (LCK), whom the participant believed to be a psychologist that was evaluating his or her performance on the tasks. Participants also were told that the videotaped speech would be evaluated by a panel of psychologists. This stressor protocol and time course of biomarker sample collection successfully induces a biobehavioral stress response (e.g. Klein et al., 2010). Blood samples were collected 15 and 45 min after the cessation of the stressor; BP and HR were recorded every 2 min throughout the stressor and recovery period. After the final blood draw, participants were debriefed about the video-taping procedure and caffeine administration; the catheter was removed, and participants were paid for their time.

Blood sample handling

For each of the three blood draws, blood was drawn into anticoagulant-free (serum) and chilled ethylene-diaminetetraacetic acid (EDTA; plasma) tubes. EDTA tubes immediately were centrifuged for 15 min at 4 °C at $1500 \times g$, and plasma samples were stored at -80 °C for later fibrinogen assessment. Serum samples were allowed to sit at room temperature for 15 min prior to centrifugation at 4 °C at $1500 \times g$ for 15 min and then were frozen at -80 °C for later CRP, cortisol, estrogen and progesterone assessment.

Assays

C-reactive protein levels were assessed using an enzyme linked immunosorbent assay (ELISA) kit developed in-house at the GCRC (DakoCytomation, Glostrup, Denmark) that has a minimum sensitivity of 3.9 ng/mL and upper range of 4000 ng/mL. Fibrinogen levels were assessed using an ELISA (Affinity Biologicals, Inc., Ancaster, Ontario, Canada); this kit has a minimum sensitivity of 0.003 mg/mL and an upper limit of 200 mg/mL. Cortisol, estradiol and progesterone levels also were assessed using commercially-available ELISA kits (Diagnostic Systems Laboratories, Inc., Webster, Texas). The cortisol kit has a minimum sensitivity of 0.1 μg/dL and upper range of 60 μg/dL. The estradiol kit has a minimum sensitivity of 7 pg/mL and upper range of 750 pg/mL. The progesterone kit has a minimum sensitivity of 0.13 ng/mL and upper range of 80 ng/mL. All assays were run in duplicate with mean values being reported and used for analyses. Duplicate test values that varied by more than 5% error were subject to repeat testing.

Design and data analyses

The study design comprised a 2 (sex) $\times$ 2 (caffeine, placebo) design. To ensure all baseline characteristics were equally distributed among groups, two-way analyses of variance (ANOVA) tested continuous data and χ^2 analyses were used for categorical data. Ethnicity data was collapsed to Whites and all minorities to meet χ^2 assumptions that no cells expected count should be less than 5. Separate repeated-measures ANOVA were conducted to test the effects of sex and caffeine on BP, HR, cortisol, fibrinogen, and CRP across the laboratory session. Separate ANOVAs were used to test any significant group X time interactions. Cortisol, fibrinogen, and CRP data were natural log transformed to normalize their distribution (Walter et al., 2012; Whetzel et al., 2006). SBP, DBP, and HR were averaged across each time period (i.e. baseline, stress and recovery) as described by Llabre and colleagues (1988). All tests were two-tailed, and criterion for statistical significance was set at alpha = 0.05. All tables and figures present means ($\pm$SEM) based on untransformed (raw) data values for clarity.

Results

Participants

The caffeine group was significantly older than the placebo group by 1.5 years [$F(1,48) = 6.25$, $p < 0.05$]; however, when controlling for age in the cardiovascular and biomarker analyses, it was not a significant predictor; the small effect sizes ranged from 0.000 to 0.040, and the results remained similar across all analyses. Therefore, age was dropped from the reported analyses.

As expected, male participants had a greater baseline SBP than did female participants [$F(1,48) = 30.54$, $p < 0.05$], but there were no sex differences for DBP or HR. No SBP, DBP, or HR baseline differences were

observed between caffeine and placebo groups (all F s 2.00, p s > 0.05). At baseline, no caffeine group or sex differences were observed for cortisol or CRP levels. At baseline, group differences were revealed for fibrinogen levels, such that the no caffeine group had significantly higher fibrinogen levels than did the caffeine group [$F(1,48) = 4.707$, $p < 0.05$].

Self-reported stress

As expected, self-reported stress changed across the laboratory session [$F(2,94) = 28.59$, $p < 0.05$]. Specifically, self-reported stress levels were higher under stress compared with baseline and recovery [$F(1,47) = 38.91$, $p < 0.05$ and $F(1,47) = 32.34$, $p < 0.05$, respectively], and baseline and recovery levels were similar. Time did not interact with sex or caffeine conditions, and there were no main effects of sex or caffeine treatment on self-reported levels of stress.

Blood pressure and heart rate measures

Table II presents mean SBP, DBP and HR values for men and women by caffeine exposure group during baseline, stress, and recovery. As expected, SBP, DBP, and HR all increased under stress and decreased during recovery [$F(2,96) = 197.18$, $p < 0.05$; $F(2,96) = 256.27$, $p < 0.05$; $F(2,96) = 80.53$, $p < 0.05$; respectively]. During stress, caffeine administration enhanced the SBP response and subsequently delayed recovery compared with no caffeine exposure [time by caffeine treatment interaction: $F(2,96) = 3.57$, $p < 0.05$]. Caffeine had no impact on DBP or HR reactivity [all F s < 1.50, all p s > .05]. Neither sex nor the interaction was significant for SBP, DBP, or HR across the laboratory session [all F s < 1.66, all p s > .05].

Serum cortisol

As expected, cortisol increased during stress and decreased at recovery [$F(2,94) = 19.62$, $p < 0.05$]. In addition, sex and caffeine independently affected cortisol production. Males produced significantly more cortisol in response to stress than females and did not return to baseline levels at recovery as did females [$F(2,94) = 4.20$, $p < 0.05$]. Caffeine administration enhanced the cortisol response to stress and delayed

Table II. Mean (± SEM) raw values for blood pressure, heart rate, serum cortisol, serum C-reactive protein, and plasma fibrinogen levels across the laboratory session by sex and caffeine treatment groups

	Males		Females		
	0 mg/kg($N = 13$)	3.3 mg/kg($N = 13$)	0 mg/kg($N = 13$)	3.3 mg/kg($N = 13$)	F (p)
Systolic blood pressure (mmHg)					
Baseline	115.58 ± 1.74	116.83 ± 2.21	106.41 ± 2.89	100.33 ± 2.15	31.54 (<0.001)[a]
Stress[b]	134.32 ± 3.13	141.14 ± 3.22	121.61 ± 4.28	120.63 ± 2.76	23.93 (<0.001)[a]
Recovery	119.42 ± 2.18	123.94 ± 2.84	107.77 ± 3.10	106.25 ± 2.35	30.83 (<0.001)[a]
Diastolic blood pressure (mmHg)					
Baseline	60.92 ± 1.64	62.08 ± 1.75	63.25 ± 1.55	59.69 ± 1.73	NS
Stress[b]	74.30 ± 2.24	78.38 ± 1.67	76.79 ± 2.36	74.26 ± 1.62	NS
Recovery	65.78 ± 1.57	68.77 ± 1.37	66.48 ± 1.67	64.92 ± 1.34	NS
Heart rate (beats per minute)					
Baseline	69.18 ± 2.57	69.71 ± 2.00	67.31 ± 2.77	65.20 ± 2.58	NS
Stress[b]	80.18 ± 3.77	78.60 ± 2.77	79.37 ± 2.91	74.75 ± 2.14	NS
Recovery	68.49 ± 2.44	70.68 ± 1.97	70.00 ± 2.57	66.27 ± 1.83	NS
Cortisol (µg/dL)					
Baseline	10.52 ± 1.16	8.27 ± 0.66	10.99 ± 1.36	12.04 ± 1.96	NS
Stress[b]	15.81 ± 2.62	17.06 ± 1.81	11.48 ± 1.01	15.88 ± 2.24	NS
Recovery	10.64 ± 1.04	12.37 ± 0.92	8.79 ± 0.79	11.97 ± 1.16	6.38 (0.015)[c]
Serum C-reactive protein (mg/L)					
Baseline	0.117 ± 0.038	0.154 ± 0.064	0.110 ± 0.044	0.158 ± 0.047	NS
Stress	0.116 ± 0.037	0.165 ± 0.073	0.116 ± 0.138	0.155 ± 0.047	NS
Recovery	0.118 ± 0.038	0.164 ± 0.072	0.113 ± 0.044	0.163 ± 0.054	NS
Plasma fibrinogen (mg/dL)					
Baseline	5.40 ± 0.54	4.17 ± 0.17	4.35 ± 0.26	4.10 ± 0.22	4.71 (0.035)[d]
Stress	4.09 ± 0.24	4.08 ± 0.17	4.15 ± 0.14[e]	4.67 ± 0.37[e]	12.45 (0.001)[c]
Recovery	4.37 ± 0.20	4.19 ± 0.14	4.16 ± 0.15	4.79 ± 0.54	3.98 (0.052)[c]

[a]Males greater than females.
[b]Stress levels greater than baseline and recovery.
[c]Caffeine group greater than placebo group.
[d]Placebo group greater than caffeine group.
[e]Females greater than males.

recovery from stress such that cortisol remained significantly elevated among men and women administered caffeine but not in the placebo group $[F(2,94) = 4.02, p < 0.05]$. The sex by caffeine interaction was not significant across the laboratory session $[F(2,94) = 0.89, \text{n.s.}]$. Table II presents baseline, stress, and recovery levels for all blood biomarkers examined.

Serum C-reactive protein

One male participant was removed from the CRP analyses because his CRP levels were identified as outliers (4 standard deviations above the overall sample mean at all 3 time points). No effect of time on CRP levels was observed across any of the study phases $[F(2,92) = 0.47, p > 0.05]$ nor were any effects of sex, caffeine, or the interaction $[F\text{s}(2,92) < 2.07, p\text{s} > 0.05]$.

Plasma fibrinogen

Significant differences in baseline fibrinogen were evident as a result of males (but not females) in the no caffeine group being elevated. This difference was not viewed as a systematic effect of caffeine administration; therefore, baseline fibrinogen levels were included as a covariate when analysing fibrinogen changes from stress to recovery and assessing differences at each time point. Figure 1 presents stress and recovery plasma levels of fibrinogen adjusting for baseline levels for all four groups.

Fibrinogen levels did not significantly change across the stress and recovery phases $[F(1,47) = 0.44, \text{n.s.}]$. Caffeine and sex did not interact with time to affect fibrinogen levels from stress to recovery $[F\text{s} < 1.29, p\text{s} > 0.05]$. However, at stress, females had elevated levels of fibrinogen compared with males $[F(1,47) = 12.45, p < 0.05]$ and participants in the caffeine condition had significantly greater fibrinogen levels relative to those in the placebo condition $[F(1,47) = 11.86, p < .05]$. During recovery, fibrinogen
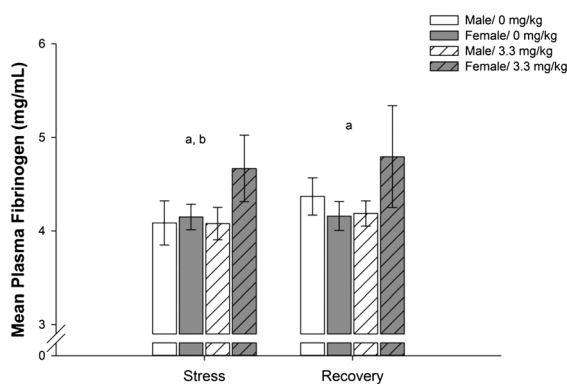

Figure 1. Mean plasma fibrinogen (mg/mL) levels (±SEM) adjusted for baseline levels by sex and caffeine treatment groups at stress and recovery. [a] Caffeine group > placebo ($p < 0.05$); [b] Females > males ($p < 0.05$).

remained elevated in caffeine participants compared with the placebo group $[F(1,47) = 3.98, p = 0.05]$; however, fibrinogen level between sexes were similar.

Discussion

Our BP and HR results confirm previous studies with men (e.g. al'Absi et al., 1997; al'Absi et al., 1998; Lovallo et al., 1991) and women (Farag et al., 2006; Hartley, Lovallo, & Whitsett, 2004). Specifically, caffeine administration resulted in a greater SBP response from baseline and remained elevated during the post-stress recovery period for both men and women. These effects were not observed, however, for DBP and HR. The stress-induced changes in cortisol levels were enhanced independently by caffeine treatment and being male relative to placebo and females, respectively. Neither CRP nor fibrinogen levels changed in response to the stressor. However, following stress, fibrinogen levels were elevated in females compared with males and in those exposed to caffeine relative to the placebo group; these findings appear to be driven by the females who received caffeine.

Regarding the DBP findings, our results differ from previous reports (Hartley et al., 2004; Lane, Phillips-Bute, & Pieper, 1998), possibly due to the habituation process that takes place in response to repeated caffeine exposure, type of BP measurement (ambulatory versus sitting) or age of participants (i.e. our sample was 7–15 years younger than prior reports). DBP has been shown to adapt to repeated caffeine exposure, where SBP appears to consistently increase in response to caffeine (Corti et al., 2002; Sudano et al., 2005). Similarly, regarding HR, caffeine-naïve subjects display significant BP and HR elevations following caffeine exposure. However, in contrast to the BP measures, HR adapts quickly to the repeated caffeine administration, although this effect may be attenuated in older populations (Sudano et al., 2005). Because participants in the current study were daily caffeine consumers, it is likely that their cardiovascular system habituated to the DBP and HR effects of caffeine prior to the laboratory session.

There are two potential mechanisms for the SBP elevations we observed. One putative mechanism involves caffeine's antagonistic effect on adenosine receptors (Julien, Advokat, & Comaty, 2011). The second mechanism is that caffeine causes increased activation of the renin angiotensin system that helps regulate long-term BP and extracellular volume in angiotensin. Caffeine and its metabolites potentiate vasoconstriction by blocking adenosine and act to enhance sympathoadrenal activation of the renin angiotensin system (Onrot et al., 1986; Robertson et al., 1981). Brown and colleagues (1993) found that caffeine increased baseline plasma levels of renin activity. Several studies suggest the hypertensive effect of caffeine involves only the disruption of adenosine's vasodilatation properties and not the activation of the renin angiotensin system (e.

g. Shinzato et al., 1994; Tanner & Tanner, 2001); however, the sample populations from these studies have some form of kidney disease. Because the present study population was healthy, caffeine's SBP effects may be the result of both mechanisms working in parallel.

Increased cortisol production in response to stress is supported by a large body of research (for review see Dickerson & Kemeny, 2004). Caffeine administration enhances the cortisol response during stress in men with and without a family history of hypertension (al' Absi et al., 1998; Lovallo et al., 1989, Lovallo et al., 1991; Lovallo et al., 1996). Lovallo et al. (2006) also reported that women without a family history of hypertension produce more cortisol in response to stress (mental and exercise) when caffeine was present. In the laboratory environment, this is the first report of an exaggerated cortisol response to caffeine administration under stress in women with a family history of hypertension.

Our data revealed sex differences in cortisol responsivity, regardless of caffeine administration; men had a significantly greater increase from baseline to stress relative to women and cortisol remained elevated at recovery compared with baseline in men, but women returned to or dropped below baseline cortisol levels. These results are in direct contrast to recent reports of no sex differences in serum (Kelly, Tyrka, Anderson, Price, & Carpenter, 2008) and salivary (Schoofs, Hartmann, & Wolf, 2008; van Stregeren, Wolf, & Kindt, 2008) cortisol reactivity. Despite this contrast, our findings add to an existing body of literature (Kirschbaum et al., 1999; Kudielka et al., 2004; Kudielka et al., 1998); suggesting men may have a greater cortisol response to psychological stress than do women.

We investigated the effects of caffeine and stress on CRP, the primary clinical blood marker of CVD risk. In our study, the addition of caffeine did not affect the relationship between stress and CRP levels in men and women with a family history of hypertension. The lack of CRP stress responsivity is not completely surprising given the healthy young population studied and data suggesting that CRP does not reliably respond to acute stress (Steptoe, Hamer, & Chida, 2007).

This study was the first to examine the combined effect of stress and caffeine on fibrinogen levels in men and women. Unexpectedly, fibrinogen levels at baseline were significantly elevated for those in the non-caffeine group compared with those in the caffeine condition. The study design (first blood draw approximately 20 min after caffeine administration) and/or the lack of strict dietary controls [high fat meal has been shown to increase fibrinogen (Blake & Ridker, 2003; Muldoon et al., 1995; von Kanel & Dimsdale, 2003)] may have contributed to baseline differences.

Our data suggests that caffeine administration significantly elevated fibrinogen 15 min following the stressor and during recovery relative to those who did not receive caffeine, which supports previous findings by Happonen and colleagues (1987). Additionally, in our data, a main effect of sex was found, with females having significantly higher fibrinogen levels than males at stress. Post-hoc tests showed that the main effects for sex and caffeine conditions appeared to be due to the higher levels in the caffeine-treated women compared with all other groups.

Participants were asked to stop consuming caffeine 4 h prior to the session starting. It is important to note that the average half-life of caffeine is 4.5 h, suggesting that participants would not be in caffeine withdrawal. However, the variability in residual caffeine levels upon arrival to the lab may have influenced the baseline level of fibrinogen or added greater variation in the biological responses to the stressor, thus limiting our ability to detect changes based on our caffeine manipulation.

A limitation of this study is the small sample size. On the basis of our data, it would require 370 participants per group to have 80% power to detect significant caffeine by sex interaction in fibrinogen responsivity to stress. To detect significant caffeine by sex interaction in CRP responsivity to stress with 80% power, the study needed to recruit 23 participants per group. In addition, the lack of strict control on the participants' lives outside of the laboratory may have affected the results. For example, there are factors known to affect CRP and fibrinogen levels, including exercise and sleep, which were not controlled in the current study. Future studies must increase sample size and measure or control these potential confounds. In addition to increasing sample size, future studies should provide an additional control condition including individuals without a family history of hypertension and could increase power by collecting repeated within-person data using a crossover design.

One methodological advantage in the present study compared with previous research was the individual titration of caffeine at the visit, on the basis of previous research by Lovallo et al. (1996) and al'Absi et al. (1998). Tailoring the caffeine dosage may be one reason why significant increases in fibrinogen level were found in the present study but not in others (Bak & Grobbee, 1990; Naismith et al., 1970). Furthermore, previous studies neither examine the effects of caffeine on fibrinogen levels in combination with stress nor have they examined the effects of caffeine on fibrinogen levels in a population with a family history of hypertension. Because previous studies neither included females nor examine sex differences within their sample, only future studies can confirm the sex difference indicated here.

This study was the first to examine changes in CRP and fibrinogen reactivity to caffeine and stress in women, a sample population previously neglected. Results from this study may indicate important sex differences in the relationship among stress, caffeine and fibrinogen. Our data show no relationship among stress, caffeine and CRP, suggesting that CRP may not be reactive to acute stress or acute caffeine administration in a young healthy population.

The data from this study add to the literature in a number of ways; it examines (1) the effects of caffeine and psychological stress on CRP and fibrinogen, (2) changes in CVD blood markers in a population with a family history of hypertension and (3) neuroendocrine and cardiovascular reactivity to stress and caffeine in females with a family history of hypertension. Finally, the relationship among caffeine, stress, sex, and fibrinogen suggests this line of research may have implications for the prevention, diagnosis and treatment of CVD. Although the necessity for replication of the current findings is unarguable, the current study suggests that the combination of stress and caffeine may be particularly detrimental for females with a family history of hypertension.

Conflict of Interest

None of the authors have competing financial interests to disclose.

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